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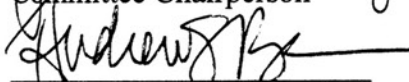
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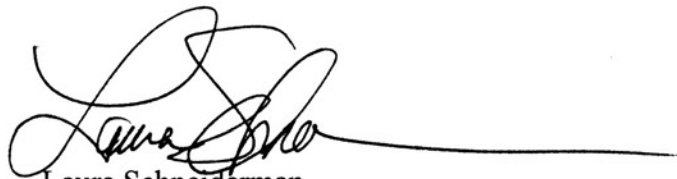

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A handwritten signature in black ink, appearing to read 'Laura Schneiderman', with a long horizontal line extending to the right.

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ABSTRACT

Title of Dissertation: The Relationship between Beta-
Adrenoreceptor Density and Immune Function
Before and After Acute Stress

Laura Schneiderman, Doctor of Philosophy, 1994

Dissertation directed by: Andrew Baum, Ph.D., Department of
Medical Psychology

Prior research has described increases in β -adrenoreceptor number caused by acute infusions of catecholamines, as well as in response to a psychologically stressful mental arithmetic task. These studies suggest that stress induced increases in catecholamines are related to changes in β -adrenoreceptor density. Decreases in lymphocyte proliferation to a mitogen in response to various psychological stressors have also been observed. However, whether β -adrenoreceptors are moderators of lymphocyte activity during psychologically challenging or stressful tasks had not been examined.

This study investigated changes in β -adrenoreceptor density and lymphocyte proliferation in response to mitogens, Con A and PWM associated with a speech task. Also, the relationship between changes in β -receptor density and lymphocyte proliferation was examined. A total of 40 subjects participated in this study.

The primary objective of the present study was to

determine whether there were psychological stress related changes in β -adrenoreceptor density and lymphocyte function, and whether changes in β -adrenoreceptor density predicted changes in lymphocyte mitogenesis. The experimental group prepared a speech for 5 minutes and then gave the speech for 3 minutes while being videotaped. The control group read simple words to themselves for 5 minutes and then read the words aloud for 5 minutes. Blood was drawn to examine mitogenic activity and β -adrenoreceptor density at four time points: baseline (twenty minutes after the catheter insertion), immediately after speech preparation/silently reading, 5 minutes after giving the speech/reading aloud, and again 30 minutes after the tasks.

Results of the study revealed that subjects giving a speech had significant increases in β -adrenoreceptor density compared to control subjects, and had significantly decreased lymphocyte proliferation to PWM but not Con A compared to controls. Most importantly, psychological stress induced increases in β -adrenoreceptor density significantly predicted decreases in lymphocyte activity to both Con A and PWM.

Although the results show an association between β -adrenoreceptor density and lymphocyte activity, β -adrenoreceptor density appears to be only one factor involved in immune modulation. Further study is needed to determine other factors involved, such as whether receptors for different hormones are also present on lymphocytes.

The Relationship Between Beta-Adrenoreceptor Density and Immune
Function Before and After Acute Stressor Exposure

By

Laura Schneiderman

Dissertation submitted to the Faculty of the Department of Medical
and Clinical Psychology Graduate Program of the Uniformed Services
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Introduction

Psychoneuroimmunologists have found that stressful events such as medical school examinations, separation/divorce, and care giving for Alzheimer's disease patients are associated with decreased immune function (Workman and La Via, 1987; Kiecolt-Glaser et al, 1984; Glaser et al, 1985; Kiecolt-Glaser et al, 1987; Kiecolt-Glaser et al, 1987). Further, immunocompromise (decreased ability to respond to a foreign substance or pathogen) associated with prolonged stress has been suggested by some to be associated with illness, cancer progression (Riley, 1981; Shavit et al, 1983), and infectious illness (Biondi & Kotzalidis, 1990; Cohen, & Williamson, 1991). The present study examined some of the mechanisms which may be involved in immune changes during stress. Many researchers have suggested that stress and its associated immune changes may be a mechanism by which life events may generate health changes. At the same time, many have turned to controlled laboratory studies to chart psychological and hormonal changes associated with acute stress (eg. Naliboff et al, 1991;

Bachen et al, 1992; Brosschot et al, 1992; Manuck et al, 1991).

Changes in immune function that occur during or after acute laboratory stressors are probably subclinical and most likely do not effect health. However, discovering the mechanisms involved in immunosuppression during acute laboratory stressors (which can be better controlled than field studies of chronic stressors) may allow interventions to be developed that are specific to these mechanisms and may prevent adverse health effects that can occur from chronic stress.

The physiological components of stress are characterized by sympathetic and hypothalamic-pituitary-adrenal axis activation with increases in heart rate, blood pressure, respiration, and levels of catecholamines (epinephrine and norepinephrine), cortisol, and other hormones (eg. Von Euler & Lundberg, 1955; Frankenhaeuser & Kareby, 1962; Shaeffer & Baum, 1984; McCubbin et al., 1983; Dess et al., 1983; Shavit & Martin, 1987). Hormonal changes associated with sympathetic nervous system (SNS) arousal (eg. epinephrine and norepinephrine) may mediate the effects of psychological factors on immunity. Lymphocyte activity is suppressed following exposure to

norepinephrine (NE) and epinephrine (E) during in vitro incubation, by infusions with E, and by increases in NE and E levels associated with psychological stressor exposure and exercise (eg. Carlson, Brooks & Roszman, 1989; Hirschberg et al, 1982; Exon et al, 1990; Faith et al, 1984; Crary et al, 1983; Manuck et al, 1991).

NE is released primarily from sympathetic neurons during stress and stimulates hepatic glycogen breakdown into glucose, selectively constricts blood vessels, and increases vascular blood flow, and increases respiratory rate (Hadley, 1984). Epinephrine has biological functions similar to NE, but is released from the adrenal medullae. These physiologic responses may be adaptive during exposure to an acute stressor, allowing an organism to prepare the body for resistance against the stressor and to respond to the stressful situation. However, prolonged activation may not be adaptive and may negatively affect health. Selye (1976) suggested that chronic stress can lead to exhaustion and disease. Similarly, acute changes in immune function may be adaptive, whereas chronic or repeated acute stress may decrease resistance against illness.

Although many stress-related hormones may be associated

with changes in immunity, catecholamine release is of particular interest during stress because these hormones appear to be the most clearly tied to stress-related immunosuppression (eg. Ernstrom & Sandberg, 1973; Yu & Clemets, 1976; Crary et al., 1983). One pathway in which catecholamines may affect immune function is via β adrenoreceptors on lymphocytes. The availability of β adrenoreceptors (ie. β adrenoreceptor density) can affect the susceptibility of lymphocytes to the influences of NE and E. The present study examined the role that changes in β adrenoreceptor density on lymphocytes play in lymphoproliferation during and after acute stressor exposure.

The remainder of this introduction will review the literature on psychological stress and the role that NE and E play in stress-related immune system alterations. In the first section, stress is defined. In the second section, the effects of stress on immune function and sympatho-medullary activity is reviewed and evidence for E and NE mediation of stress-related immune system change is considered in detail. Finally, the mechanisms involved in catecholamine- (ie. NE and E) induced immune changes will be

examined.

Defining Stress From a Psychobiological Perspective

The use of the term stress has been ambiguous over the years and has meant different things to different researchers. In the 18th century Thomas Young suggested that stress is a response inherent in an object which is extracted by an external force (see review from Engel, 1985). This physical concept was later adopted by a number of biologists, including Claude Bernard a 19th century physiologist who defined stress as an adaptive reaction to external stimuli (see review from Engel, 1985). He described stress as the body's attempt to return to a homeostatic state after equilibrium is disturbed. When the adaptive reaction was insufficient to resist the deleterious stimulus or damage to the body due to the actual physiological response, the result was disease. Many years later Selye (1974) also maintained that stress is best defined as a response to an aversive stimulus. However, he included an important concept, that all noxious stimuli elicit a specific physiologic pattern. Regardless of what the agent was, the stress response was

physiologically the same (eg. changes in the adrenal and thymus glands).

In order to describe the processes involved, Selye developed the General Adaptation Syndrome (GAS). The three stages of response in his GAS model include an **alarm reaction**, and stages of **resistance**, and **exhaustion**. During the alarm reaction, the organism first perceives the noxious stimulus, and prepares to resist it. Adrenal-cortical activity increases and when corticosteroid levels have risen, the stage of resistance is initiated. Here adaptation is usually achieved and the negative stimulus is successfully resisted. If these reactions reoccur a number of times or are prolonged due to failure to remove the noxious stimulus, physiological damage to the organism may occur. This could result in exhaustion, where the adaptive reserves are diminished, resistance is not achieved, and disease may result. Mason (1975) disagreed with Selye's nonspecific response theory, suggesting that different patterns of norepinephrine, epinephrine and cortisol released into circulation were associated with different stressors. In addition, Mason (1975) was concerned with the role of

psychosocial factors suggesting that awareness of the negative stimuli is necessary for a stress response.

In contrast with those who thought of stress as a response, Cannon (1913) described stress as an external force, such as an emotion-eliciting stimulus (eg. a dog barking at a cat). He suggested that emotional stress could lead to physiological changes and could result in medical problems. Lazarus (1966) suggested that stress should be used as a general label incorporating the entire process which includes the stimuli producing the response, the reaction itself. Lazarus (1975) described a primarily psychological process, emphasizing the role of cognitive appraisal and perception in response to the stressor. Like Mason, Lazarus suggested that an organism must perceive a situation as threatening in order to experience stress. Frankenhaeuser and her colleagues (eg. 1962, 1971) also demonstrated in a number of studies that psychological factors play an important role in stress. However, physiological components of stress were examined in response to these psychological factors by observing responses such as NE and E to psychological stressors. In order to minimize confusion, in this

paper the term "stress" will be used to describe the entire process outlined by Lazarus, including the stimulus, response and intervening variables.

Effects of Stress on Sympathomedullary Activity

Eighty percent of circulating NE comes from sympathetic nerve terminals innervating vascular smooth muscle (Guyton, 1991). The remaining 20% of NE and 100% of E are secreted from the adrenal medulla. Two types of adrenoceptors exist: α - and β - adrenergic receptors (adrenoceptors). The α -adrenoceptors respond to a potency ranking of $E > NE > \text{isoproterenol (ISO)}$ while β -adrenoceptors respond to $ISO > E > NE$. Generally, stimulation of α -adrenoceptors elicits smooth muscle contraction and stimulation of β -adrenoceptors evokes relaxation. There are exceptions, however; for example, E-induced stimulation of β -adrenoceptors on the heart results in a *contraction* of heart muscles which increases the force and the rate of the heartbeat. In addition, the α -adrenoceptors on vascular smooth muscle are stimulated during stress, resulting in blood being shunted from skin, connective tissue,

mucosa and kidneys. At the same time, smooth muscle of coronary arteries, and skeletal muscle (which have β -adrenoreceptors) “relax” to receive the blood (Guyton, 1991). Thus, depending on which adrenoreceptors are present, NE or E can either contract or relax smooth muscle.

Both α - and β - adrenoreceptors have two pharmacologically different subgroups. α -adrenoreceptors consist of α_1 -adrenoreceptors which are associated with smooth muscle vasoconstriction and α_2 - adrenoreceptors which mediate feedback inhibition of NE (Hadly, 1984). β_1 -adrenoreceptors are found in adipose tissue and heart muscle with a potency of ISO \gg E = NE while β_2 - adrenoreceptors (whose stimulation causes bronchodilation and vasodepression) have a potency response of ISO $>$ E \gg NE. Adrenoreceptors are not only found on vascular smooth muscle but have also been observed on immune cells and platelets (Barns, 1981).

Primarily β_2 - adrenoreceptors are thought to be found on lymphocytes (eg. Graafsma et al, 1987; Maisel et al, 1991; Van Tits et al, 1990; Khan et al, 1986). For β_2 - adrenoreceptors , E is 10-30

times more potent than NE (Barns, 1981; Brodde, 1986). Therefore, increased levels of E resulting from stress may have a greater affect on immune function than increased NE levels.

Elevated NE and E levels during and after stressful events are evident for both acute and chronic stressors. Though NE and E frequently increase simultaneously, there may be variations in excretion patterns of E and NE during different types of acute stressful situations. These differences in excretion patterns may be important for eliciting immune system changes during different types of stressors, since lymphocytes may respond to NE and E differently. For example, one study examined E and NE levels at baseline, during public speaking, and during stair climbing exercise (Dimsdale & Moss, 1980). Plasma levels of E more than doubled during public speaking, whereas during stair climbing E increased by only 50%. On the other hand, plasma levels of NE almost tripled during exercise, but did not significantly increase during public speaking (there was a trend, however). Both stressors elicited NE and E elevations, however the stressor with the stronger physical component (stair climbing), caused larger NE responses compared to

the speech stressor which caused a larger E response. Speaking is a somewhat physical act (moving the mouth, head, hands etc.) and climbing stairs may have a psychologically stressful component, making it difficult to separate physical and psychological elements of stress completely. Ward and her colleagues (1983) examined plasma E and NE responses to a series of stressors, mental arithmetic, handgrip and knee bends, venipuncture and cold pressor, and a nonstressful medical procedure (blood pressure measurements). E and NE significantly increased in response to the stressors but E was observed to increase to a greater extent to the psychological stressor (mental arithmetic). These studies suggest that there are differences in hormone levels depending on whether the task involves physical activity and/or sensations or are more psychologically challenging. Thus, speech and mental arithmetic, which are assumed to be psychologically challenging may elicit greater increases in E compared with NE.

During chronic stressful situations, catecholamine levels appear to remain high as long as events remain unpleasant (Eliasson, 1984). However, there are few studies in the literature examining

the effects of chronic stress on catecholamine levels. In one study, subjects living near Three Mile Island showed higher levels of urinary catecholamines (collected over 15 hours), seventeen months after the nuclear accident than did control subjects living at other sites (Baum, Gatchel & Schaeffer, 1983). In another study, individuals who were unemployed for longer than two months exhibited higher levels of urinary E and NE. However, urine was collected once during the session and not collected over a number of hours (Fleming, Baum, Reddy & Gatchel, 1984). In a third study, plasma levels of NE and E were measured in medical students. They had an increase in plasma E and NE over three months prior to medical board examinations (O'Donnell et al, 1987). Urinary measures of catecholamines collected over a number of hours and plasma samples taken over a number of months may better reflect chronic stressors than plasma or urine measures taken once, which instead usually measure acute changes which may be unrelated to the study (eg. heavy traffic on the way to the laboratory session, an argument with a spouse). These studies attempted to examine chronic stressors since the effects on the individuals appear to last

for a prolonged period of time.

Taken together, these studies suggest that stressors may elicit elevations in E and NE levels for various lengths of time. The patterns of elevation in E and NE appear to be task dependent and may be associated with physical or psychological components. With prolonged exposure to stressful events (and therefore extended physiological responses) there may be an effect on health. Immune system alterations due to stressor exposure may affect health. Since decreases in immune function as a result of stressor exposure are thought to be involved in infectious illness (Biondi & Kotzalidis, 1990; Cohen & Williamson, 1991) and cancer progression (Riley, 1981; Shavit et al, 1983), stress related immune system alterations may have important health implications.

Stress, Catecholamines, and Immunity

Various immune measures have been used to observe changes in immune responses during stress. Some measures look at immune function, ie. the ability of immune cells to combat foreign or "invading" substances. Functional measures performed in vitro

examine the ability of white blood cells to respond to specific substances in a cell culture outside of the body, simulating how these cells fight pathogens while in the body. Other measures examine the numbers of total immune cells, and individual immune cell subsets, and the ratios of these subsets. These measures do not directly examine the ability of the immune cells to respond to a pathogen. However, immune cell numbers may have an overall effect on how an individual fights against illness or disease, since sheer quantity may play a role in whether a pathogen is eliminated.

One measure of immune function used during and following acute psychological stressor exposure documents changes in the ability of T and B lymphocytes to proliferate in vitro in response to a mitogen (a substance which stimulates cells to divide). Mononuclear leukocytes (MNLs) consisting of T, B, and natural killer (NK) cells and macrophages are isolated from whole blood and are exposed in vitro to mitogens such as concanavalin A (Con A), pokeweed mitogen (PWM) and phytohemagglutinin (PHA) which are plant lectins. T and B lymphocytes view these mitogens as foreign proteins and respond to their presence by dividing

(lymphoproliferation), producing more T and B lymphocytes. In order to measure T and B lymphocyte proliferation, tritiated thymidine (^3H -TdR) is added to the cell culture medium and is incorporated into the T and B cells' DNA during mitosis (cell division). Thus, the extent of lymphoproliferation is directly measured by the incorporation of ^3H into the lymphocyte DNA.

Enumeration of immune cells in circulation and ratios of subclasses of immune cells are measured by detecting protein markers on the cell surface which are specific to that cell. For example, T helper cells can be identified by the presence of the protein CD4 on their membrane.

Catecholamines as Mediators Between Stress and Immune Cell Number

Acute stress in humans appears to affect immune cell numbers. Studies found increases in CD8+ T cells and NK cells in response to both the Stroop task as well as mental arithmetic stressors and an interpersonal stressor (Naliboff et al, 1991; Bâchen et al, 1992; Brosschot et al, 1992). However, other studies have not

found increases in CD8+ T cell numbers (eg. Sieber et al, 1992; Knapp et al, 1989) or NK cells (eg. Sieber et al, 1992) during acute stress. The reasons for these discrepant findings may include the amount of time elapsed after the stressors when immune measures were taken, differences in experimental method and task differences. Also, hormones such as catecholamines were not measured, so mechanisms involved in the immune system changes could not be determined.

Catecholamines may function as moderators of the stress response by altering immunity during or following stressor exposure. NE and E have been associated with several changes in the immune system, the most basic of which is a change in numbers of circulating white blood cells. Sympathetic innervation in the thymus and spleen (Williams et al, 1981) as well as adrenergic receptors on lymphoid tissue (Ernstrom & Sandberg, 1973) are likely mechanisms for these effects.

The role of adrenergic activity was examined by Ernstrom and Sandberg (1973) who reported that both NE and isoproterenol (ISO) (a β -adrenoreceptor agonist) injections in rats stimulated a rapid increase in numbers of lymphocytes and granulocytes in splenic

venous blood. However, there were also greater numbers of lymphocytes in the arterial splenic blood in the injected animals than in control animals, suggesting that some lymphocyte migration originated from sites other than the spleen. Changes in blood flow were not believed to be responsible for the increase in venous white blood cells from the spleen since injections with either drug changed blood flow only slightly compared to control animals.

Yu and Clements (1976) also found increases in numbers of T and B lymphocytes in peripheral blood in humans ten minutes after injection of E (from preinjection levels) compared to subjects injected with saline. This increase may not have been due solely to the release of cells from the spleen: Three of the subjects had undergone splenectomies more than two years before the study and these participants also exhibited increased numbers of lymphocytes after injection of E. Yu and Clements (1976) ruled out cell proliferation as an alternative mechanism for increased cell numbers because proliferation of cells to the mitogen phytohaemagglutinin (PHA) did not change after the injection. Instead, they suggested that other lymphoid organs might also be

influenced by catecholaminergic activity. Other studies have also found evidence that cell proliferation decreases following administration of E, once again suggesting that increases in cell number in response to catecholamines is not due to cell proliferation but to the release of cells into circulation (Crary et al, 1983).

Studies which measured catecholamines and immune cell numbers have observed that catecholamine increases associated with psychological stressors and exercise are usually related to increased immune cell numbers. In one recent study, in response to Stroop and mental arithmetic tasks, catecholamine and cardiovascular responses were related to increases in CD8+ and NK cells (Manuck et al, 1991). In another recent study dynamic exercise, bicycle ergometry, was also associated with increased NE, E, CD8+ and NK cells, with a positive correlation observed between increases in E and circulating lymphocytes (Maisel, Harris, Rearden & Michel, 1990). Lymphocytosis, elevations in lymphocyte number, also occurred in response to exercise on a treadmill with largest increases in CD8+ and NK cells (Murray et al, 1992). Augmentation of circulating cells during acute adrenergic activation suggests an

immunoregulatory effect of the sympathetic nervous system in stressful situations (Landmann et al, 1984).

While acute stress appears to involve elevated catecholamines that are associated with increased immune cell numbers, chronic stress also is characterized by elevated E levels, but these have been related to decreases in cell numbers. In one study, people living near the Three Mile Island (TMI) nuclear power plant were compared to individuals living about 80 miles away (McKinnon et al, 1989). More than six years after the accident at TMI, subjects still exhibited signs of psychophysiological stress (eg. increased symptom reporting, elevated resting blood pressure and catecholamine levels relative to controls). They also had significantly lower numbers of B lymphocytes, NK cells, and CD8+ cells. Numbers of lymphocytes were negatively correlated with urinary levels of E ($p < .02$) and urinary NE levels were negatively related with numbers of NK cells ($p < .05$). These data suggested that stress associated with the TMI accident and its aftermath were associated with decreased numbers of cells (with catecholamines related to changes in numbers of cells), though possible radiation

effects on immune cell numbers could not be evaluated or ruled out. Similarly, caregivers' of Alzheimer's disease victims had significantly lower percentages of total T lymphocytes, and CD4+ cells, although CD8+ and NK cells were not different from controls (Kiecolt-Glaser et al, 1987). However, in another study examining bereavement in individuals experiencing the death of a spouse, there were no observed differences in cell numbers (Bartrop et al, 1977). These studies did not measure catecholamines and therefore mechanisms involved in stress and immune cell number could not be determined.

Thus, research suggests that during acute stress catecholamines may be involved in mobilization of white blood cells into the blood stream from the spleen and other lymphoid tissue (Ernstrom & Sandberg, 1973). This may be adaptive since it might prove beneficial to have increased numbers of cells in circulation during or immediately after major activity or encounters with stressors. During acute stress, exercise, and catecholamine infusions, the largest increases in immune cells are in numbers of NK and CD8+ cells (although lymphocytosis has been indicated

suggesting an increase in all immune cells). It may not be a coincidence that NK and CD8⁺ cells also have the largest number of β -adrenoreceptors (Khan et al, 1986), suggesting that catecholamines may have the greatest effects on these cells. Acute increases in NE and E may stimulate an increased release of cells from lymphoid tissue by stimulating α - and β - adrenoreceptors in the spleen, thymus, and lymph nodes.

During chronic stress, however, there appears to be a decrease in lymphocyte numbers which has been related to chronic increases in NE and E levels (McKinnon et al., 1989). This may be due to a down regulation of adrenoreceptors on lymphoid tissue, since prolonged elevations in catecholamines is suggested to reduce the number of adrenoreceptors (Barns, 1981). However, this has not been examined for its relationship with stress.

Catecholamines as a Mediator Between Stress and Immune Function

Increasing numbers of lymphocytes in circulation following injections of NE and E most likely reflect cell recirculation from different organs or storage sites in the body. These may be

important immunological changes, but the ability of these cells to function while at rest or during exposure to an antigen or mitogenic stimulus is also important to prevent or combat illnesses caused by foreign invading substances. Together, these kinds of indices define the overall ability of the organism to combat disease. A compensatory decrease in ability to combat a pathogen, for example, would negate some of the "enhancement" of overall function one might expect to see with large increases in numbers of cells.

A number of studies have examined changes in immune cell function during and after acute stress, and it has been suggested that individual differences in sympathetic reactivity and catecholamine levels during stress may be a factor in immune function. For example, in response to Stroop and mental arithmetic, proliferation of T lymphocytes to PHA decreased (along with an increase in CD8+ cells), but only in subjects exhibiting elevated catecholamine and cardiovascular reactions (heart rate, systolic and diastolic blood pressure) (Manuck et al, 1991). Another study used a combat surgery video tape and a memory test of the film as an acute stressor (Zakowski, Deal, McAllister and Baum, 1992). Lymphocyte

proliferation to Con A was significantly lower among subjects in the stressor condition compared to control subjects. Individuals in the experimental group who had the largest changes in systolic and diastolic blood pressures during the film also showed significantly less lymphocyte proliferation to Con A than did lower responders or controls. Catecholamines were not measured during this study and so the potential mechanisms involved could not be evaluated.

In order to examine the role that catecholamines play on immune cell function, Crary and his colleagues (1983) investigated T and B lymphocyte proliferation to mitogen stimulation after injections of E in humans. Lymphocyte responses to PWM and PHA were reduced for up to an hour after injection. Responses to Con A were only reduced significantly 15 minutes after injection of E. The largest inhibition of lymphocyte proliferation was found for PWM, a B cell mitogen (Crary et al, 1983). Others have also observed reduced T and B cell activity in response to β -adrenergic agonist administration (eg. Carlson, Brooks & Roszman, 1989; Van Tits et al, 1990). These data suggest decreased cell proliferation resulting from injections of β -adrenoreceptor agonists.

Blocking the action of catecholamines with a β -antagonist should negate the effects of catecholamines on immunity if immune cell activity operates through β -receptor mediated regulation. In one study, oral administration of the β -adrenergic antagonist propranolol to resting subjects was associated with enhanced Con A-stimulated lymphocyte proliferation (Maisel et al, 1991). In a similar study oral administration of propranolol blocked changes in immune activity associated with treadmill exercise by minimizing both the increase in numbers of CD8+ and NK cells, and minimizing the decrease in Con A stimulated interleukin-2 receptor expression and T cell proliferation (Murray et al, 1992). However, propranolol is a non-selective (beta 1 & beta 2) β -blocker that penetrates the CNS readily and therefore, it cannot be determined whether effects obtained by the use of propranolol are central or peripheral.

Effects of NE and E on immune function during stress were also examined in response to a conditioned aversive stimulus (CS) in rats (Luecken & Lysle, 1992). The beta 2-receptor antagonist ICI 118,551 administered prior to presentation with the CS reduced the

inhibitory effects of stress on lymphoproliferation to Con A which is a T-cell mitogen, suggesting that stress related immunosuppression was related to circulating levels of catecholamines. However, CS-induced suppression of LPS (lipopolysaccharide, a B-cell mitogen) induced B-cell proliferation was not attenuated by β -antagonists, suggesting a lack of association between NE or E and reduced B-cell activity due to stress. In another study however, there was a relationship between B-cell activity and NE and E (Crary et al, 1983). Discrepant findings between these studies may again be due to many factors including different stressors, laboratory techniques, and timing of blood draws.

Thus, one consequence of acute stress appears to be changes in numbers of immune cells and lymphocyte subpopulations. Also, cellular function may be affected by acute stress and is associated with a decrease in cell proliferation to mitogens. Sympathetic activity and catecholamine levels may be involved in some of these immune changes, since individuals who responded to stressors with higher cardiovascular and catecholamine reactions evidenced suppressed immune function.

More prolonged stressors also appear to cause diminished lymphocyte function. Bereaved subjects exhibited significantly lower lymphocyte responses to PHA and Con A six weeks after loss of a spouse than did control subjects (Bartrop et al, 1976). A prospective study of 15 husbands of women with advanced breast cancer also showed reduced lymphocyte proliferation to Con A, PHA and PWM during the first two months of bereavement compared to before the death of their spouses (Schleifer et al, 1983). Marital quality and separation/ divorce has also been studied for its effects on immunity (Kiecolt-Glaser et al, 1987) and poorer marital quality was related to higher levels of depression and lower T lymphocyte proliferation to Con A and PHA. The length of separation was also significantly and positively related to lymphocyte response to PHA. Similar findings have been reported for divorced or separated men (Kiecolt-Glaser, 1988). Relationships between NE and E, and lymphocyte proliferation during chronic stress were not examined in these studies; further investigation is needed.

Catecholamines as Mediators Between Stress and Immune

Cell Morphology

To determine a receptor's characteristics (for any pharmacologic or hormonal agent) or to determine whether a receptor is present on a cell type at all, several experiments may be performed. Three experiments, saturation, kinetics, and displacement experiments are used to determine the characteristics of individual receptors on cells: Generation of a saturation curve requires three steps. First, a radiolabeled agonist or antagonist at different concentrations is incubated with the cells to determine total binding. The "hot" ligand can bind to receptor (specific) sites as well as non-receptor (non-specific) sites. There are a finite number of specific sites and an infinite number of non-specific sites. Total binding is the combination of both. Next, another agonist or antagonist is then added to the culture which is not radiolabeled and is at a single concentration. This ligand competes with the "hot" ligand and binds to the specific sites. Thus, what are observed from this second step is radiolabeled non-specific sites. Finally, radiolabeled non-specific binding is subtracted from total binding to

determine **specific binding** at different concentrations of "hot" ligand. If a receptor exists on this type of cell the curve should appear as a rectangular hyperbola, with ligand concentration on the abscissa and specific binding on the y-axis. This is a non-linear relationship and K_d (the point where dissociation and binding reaches equilibrium) and B_{max} (the maximal amount of specific binding) cannot be easily determined by graphical analysis. However, the saturation curve can be transformed to give a linear relationship with a Scatchard (Rosenthal) plot. The parameters in the equation are represented by B (receptors bound) and F (receptors not bound, concentration of ligand), and the equation for the Scatchard plot is $B/F = -1/K_d(B) + B_{max}/K_d$. Using the Scatchard plot the K_d is the negative reciprocal of the slope of the line. Receptor density (B_{max}) can be determined by using a Scatchard plot to find the abscissa intercept (ie. when $B/F=0$), which is the maximal amount of specific binding.

A second type of experiment which is often done in receptor binding studies is a kinetic experiment where binding is determined as a function of time. Specific binding is determined by

adding a constant amount of radioisotope bound to an agonist or antagonist with another nonradioactive antagonist or agonist with cells. Cultures are then incubated for different periods of time. K_d is found by determining the forward association (k_{+1}) of the free ligand (L) to the unbound sites (R) and their reverse or dissociation (k_{-1}); $K_d = k_{-1} / k_{+1}$.

Displacement or inhibition experiments are frequently used to determine the potency of agonists or antagonists for a receptor. The concentration of the radioisotope antagonist or agonist (L) remains constant and while the concentration of the nonradioactive or unlabeled antagonist or agonist (I) varies. As I increases, RI will increase, thus reducing RL due to a decrease in R. K_i is the equilibrium dissociation constant, defined as $K_i = (R)(I)/RI$. Often the characteristics of drugs are described in terms of the IC_{50} , which is the concentration of I which inhibits 50% of the binding of RL.

β -adrenoreceptors on lymphocytes have been found by a number of researchers via radioligand binding techniques, such as those described above (eg. Graafsma et al, 1987; Maisel et al, 1991;

Van Tits et al, 1990; Khan et al, 1986). Lymphocyte β -adrenoreceptors were initially studied because alterations in β -adrenoreceptors on lymphocytes circulating in the blood stream in humans were observed to reflect alterations in functional responsiveness and density of corresponding myocardial and bronchial β -receptors (Brodde, 1986). Thus, examining changes in β -adrenoreceptor function on lymphocytes in humans could explain possible β -receptor disorders, since human tissue was not readily available (Watanabe, Lai & Yoshida, 1981). More recently, β -adrenoreceptors on lymphocytes have become of interest in their own right, since their alteration may affect immune function.

As described earlier there are two types of adrenoreceptors, α - and β - and each have two subtypes, α_1 , α_2 and β_1 , β_2 . However, only β_2 -receptors are thought to be found on lymphocytes (eg. Graafsma et al, 1987; Maisel et al, 1991; Van Tits et al, 1990; Khan et al, 1986). β_1 -adrenoreceptors are stimulated equally by E and NE, but for β_2 -adrenoreceptors E is 10-30 times more potent than NE (Barnes, 1981; Brodde, 1986). Therefore, E most

likely has a more potent affect on lymphocytes than NE. It stands to reason then, that increases in E from psychological tasks such as speech and mental arithmetic compared to physical tasks (Dimsdale & Moss, 1980; Ward,1983) would have a greater effect on lymphocytes.

Infusions of ISO and E (β_2 -agonists) produced increases in lymphocyte β_2 -adrenoreceptors, as well as increased cAMP accumulation, whereas NE did not produce this effect (Van Tits et al, 1990). In the same study pretreatment with the β_2 -selective adrenoreceptor antagonist ICI 118,551 negated the effects of ISO and E, whereas bispralol (a beta1-selective adrenoreceptor agonist) did not attenuate increases in either parameter. This may suggest that lymphocytes have β_2 -adrenoreceptors and not β_1 -receptors.

Stimulation of β_1 - and β_2 -receptors stimulate adenylate cyclase, a membrane bound enzyme, which results in an increase in cyclic AMP (cAMP) accumulated within the cell (Sutherland, Robison & Butcher, 1968). Cyclic AMP is one of the second messengers for lymphocytes as well as other cells. It has been suggested that β -

adrenergic agonists which stimulate cAMP production in lymphocytes lead to inhibition of some lymphocytic activity (Murray et al, 1993) by virtue of cAMP activity.

Catecholamines may induce changes in the activity of lymphocytes through their actions on β -adrenoreceptors on lymphocytes (Carlson, Brooks & Roszman, 1989; Van Tits et al, 1990). Numbers of receptors may either enhance or blunt these effects by increasing or decreasing access of catecholamines to the cell (the more receptors, the more chances for catecholamines to bind to the cell, and the more likely that the cell will generate cAMP which will influence the cell's activity). In addition, β -receptor density appears to be regulated by catecholamine levels; acute elevations in β -adrenergic agonists (eg. isoproterenol, E) increased lymphocyte β -adrenergic receptor density (Van Tits et al., 1990; Graafsma et al, 1990), and this effect was reversed with the β -2 antagonist ICI 18,551 (Van Tits et al, 1990).

Increased β -receptor density associated with catecholamine elevations may also reflect subpopulations of cells

released from lymphoid tissue with increased β -receptors on them.

As noted earlier, CD8+ cells have significantly more β -adrenoreceptors than CD4+ cells (Khan et al, 1986) and as reviewed earlier, increases in numbers of peripheral blood lymphocytes with a large increase of both CD8+ and NK-cell numbers are associated with elevated E levels (eg. Manuck et al, 1991; Maisel et al, 1990; Murry et al, 1992).

With prolonged infusions of β -agonist, however, decreases in number and responsiveness of β -adrenoreceptors on human lymphocytes are generally found (Beckeringh et al, 1987; Maisel et al, 1987). Van Tits and his colleagues (1990) suggested that downregulation (by decreasing β -receptor numbers) of receptors may protect cells against the antiproliferative effects of chronic exposure to β -adrenoreceptor agonists. While increased receptor density during acute exposure to β -agonists may be explained by a release of cells such as CD8+ cells from lymphoid tissue which have greater numbers of receptors, extended catecholamine exposure resulting in a decrease in β -adrenoreceptor density may also be

explained by recirculation of subpopulations of cells. During chronic stress, subpopulations of cells with greater β -adrenoreceptor densities (eg. CD8+, NK) decrease (McKinnon et al, 1989). It may be that cells with increased β -receptors on them may be redeposited back into lymphoid tissue during prolonged stress.

Some researchers have suggested that a reduction in numbers of β -receptors over time due to exposure to β -agonist is not the result of recirculation of immune cells but that sequestering of receptors within the cell occurs with prolonged exposure to a β -agonist. Thus, by reducing its number of surface β -receptors, the cell becomes desensitized to the effects of catecholamines. For example, incubating cells with ISO for 20 minutes led to a decrease in cell surface receptors, measured with a hydrophilic radioligand that is unable to enter the cell (DeBlasi, Lipartiti, Motulsky, Insel and Fratelli, 1985). However, there was not a decrease in overall receptor number, measured by using a radioactive lipophilic ligand which is able to cross the cell's membrane, and therefore can bind to receptors both on the surface, as well as within the immune cell.

This may suggest that surface receptors were drawn into the cell where the receptors then became inactive.

In another study, prolonged oral administration of the β -agonist terbutaline also decreased β -receptor number, as well as cAMP accumulation in NK cells, CD4+, and CD8+ cells (Maisel et al, 1989). The reduction in β -receptor numbers was greatest in the CD8+ cells and least in the CD4+ cells. Biphasic responses (eg. an increase and then a decrease in receptors) appear to occur not only in response to beta agonists but also with exposure to mitogenic stimuli. T cells in the presence of IL-2 (a cytokine secreted from T cells to stimulate other T cell activity) and PHA showed a biphasic response in β -receptor density and cAMP content, with an initial increase in density of β -receptors followed by a decrease (Korichneva & Tkachuk, 1990). These studies suggest that immune cells exhibit a biphasic pattern of function. At the beginning of stressor exposure there may be an increase in β -receptors which is associated with a decrease in function, whereas after a period of time (which has not been established in the literature) of stressor

exposure, there may be decrease in surface β -receptors which may be associated with a restoration of immune function.

It has been suggested that the numbers of cell surface receptors appear to be proportional to intracellular accumulation of cyclic AMP (cAMP) (Khan et al, 1986). Levels of cAMP have been related to cellular function. Binding of hormone to its membrane receptor often results in the activation of one or more nucleotide-cycling enzymes located at the inner surface of the membrane. Adenylate cyclase converts ATP to cAMP, and enzyme phosphorylation leads to a cascade phenomenon whereby activation by phosphorylation of the first enzyme allows this enzyme to act as a kinase to phosphorylate a second enzyme and so on. At each step, amplification occurs producing a response increasingly greater in magnitude (Hadley, 1984). Thus, E stimulates the beta receptors, which activate cAMP production, which amplifies the signal that E imparts to affect immune cell function. This suggests that with increased receptor density E has greater access to modulating cellular activity. Accumulation of cAMP, associated with ISO infusions is associated with a decrease in lymphocyte proliferation

to mitogen (Carlson, Brooks & Roszman, 1989). Thus, increases in β -receptors would amplify the suppressive effects of cAMP.

Mitogen stimulation decreases intracellular cAMP, whereas cAMP content is augmented with ISO, and can be reversed with the administration of a β -2 antagonist (Van Tits et al, 1990). However, when cells were incubated with both PHA and ISO together, there was a 2 to 4-fold increase in cAMP synthesis compared with ISO alone (Carlson, Brooks & Roszman, 1989). This may suggest a synergistic mechanism, where ISO increases cAMP alone but when immune cells are activated by mitogen ISO further increases cAMP and thus further decreases function. In splenectomized patients, infusions of ISO had little influence on mitogenic stimulation (Van Tits et al, 1990), suggesting that elevations in β -agonist in unsplenectomized individuals stimulate the splenic release of lymphocytes with greater β -adrenoreceptor density. This would increase β -receptor stimulation and, therefore decrease mitogen responses.

In addition to infusions of catecholamines, psychological

and exercise stress have been related to β -adrenoreceptor density changes on lymphocytes. Performing a mental arithmetic task was associated with increased β -receptor density on lymphocytes which was related to an increase in E and NE (Graafsma et al, 1987). In a follow-up study, there was a significant positive correlation between E and β -receptor density on lymphocytes in response to mental arithmetic which was blunted after adrenalectomy (Graafsma et al, 1990). This suggests that removing hormones secreted from the adrenals will decrease the affects of stress on β -receptor density. Studies have also observed increases in receptor density on lymphocytes in response to exercise (eg. Brodde, Daul & O'Hara, 1984; Middeke, Remien & Holzgreve, 1984; Graafsma et al, 1990). In an additional study, Transcendental Meditation was associated with decreased lymphocyte β -adrenoreceptors (Mills et al, 1990). However, whether meditation attenuated the effects of stress on β -receptor number was not addressed. Whether receptor density corresponds to function before, during, and after stressor exposure has not been examined.

Thus, β_2 -adrenoreceptors are found on lymphocytes and changes in their numbers have been associated with circulating β -adrenoreceptor agonist levels. β -antagonists have been observed to block these changes in β -receptor number and, increased receptor number may be associated with circulating subpopulations of cells, such as CD8+ and NK cells. With prolonged elevations in β -receptor agonists receptor density may decrease either due to recirculation of cells or sequestering of β -receptors. Also, numbers of β -receptors have been associated with cAMP accumulation and immune function with infusions of catecholamines, however such an association has not been made during exercise or psychological stress.

Additional Hormone Receptors on Lymphocytes

Additional stress-related hormone receptors may also be found on immune cells, however, the extent of their role and the role of the hormones which bind to these receptors on immune function during stress is not clear. One example is opioid receptors. On the

one hand, receptor antagonists (eg. naltrexone) have been found to negate the decrease in immune function produced by stress-induced opioid elevations (Shavit et al, 1985) suggesting the existence of an opioid receptor. On the other hand some researchers have suggested that human lymphocytes do not even have opioid receptors (Mendelsohn, Kerchner, Culwell & Ades, 1985). In another study, in vitro concentrations greater than 1 mM of morphine (a high dose of an opioid agonist) resulted in a dose-dependent suppression of proliferation of lymphocytes to a mitogen, and co-incubation with naltrexone did not negate these immune effects (Bayer, Gastonguay & Hernandez, 1992). Thus, in vitro inhibitory effects of morphine may only occur at high concentrations and may not be opioid-receptor mediated. In a conditioning study, mice were immunized with C57BL/6 spleen cells in order to stimulate NK cell activity. The immunization was paired with camphor. Naltrexone blocked the conditioned response, however quaternary naltrexone which does not penetrate the blood-brain barrier did not (Hiramoto et al, 1993). These studies may suggest that central opioid effects are related to immune responses and that when the opioid influence is removed

centrally with naltrexone, the effects on immunity are blocked.

However, endogenous opioid levels are frequently not measured, and peripheral opioid levels are difficult to interpret. Therefore the question of whether opioids (centrally or peripherally) caused conditioned immunosuppression cannot be answered at this time.

Determining the role of glucocorticoids and their receptors on immune cells are equally complicated. In one study, human T cells, large granular lymphocytes and neutrophils were examined for differences in glucocorticoid receptor density and affinity (K_d) (a dissociation constant, depicting the ability of a hormone to dissociate from a receptor at equilibrium) (Katz, Zaytoun & Lee, 1985). All immune cell types were found to have a single type of corticosteroid receptor. However, T cells had significantly fewer receptors with lower K_d than the other cells. This suggests that T cells in humans are less responsive to glucocorticoids. In another study, Ru-486, a glucocorticoid antagonist was chronically administered to healthy male subjects. There were no changes in total lymphocyte numbers, T-, B-, and NK cell subsets, NK cell activity, or lymphocyte proliferation to a mitogen. Other studies

have found relationships between glucocorticoids and suppressed immune function. However, many of these studies used synthetic glucocorticoids in order to find immunosuppression. In one study, when metyrapone was incubated with cells there was an inhibition of human T-cell proliferation to viruses (Hirschberg et al, 1982). Another study found that CD8+ cells from mice decreased in proliferation to a mitogen following administration of saturating concentrations of dexamethasone (Gillis, Crabtree & Smith, 1979). Ross and his colleagues (1982) however, observed that cortisol and corticosterone appear to have relatively low affinity to receptors compared to synthetic glucocorticoids. Therefore, results from studies using synthetic glucocorticoids should be examined carefully before drawing conclusions on the role that glucocorticoids play on lymphocyte regulation. This and other findings suggest that glucocorticoid receptors most likely exist on lymphocytes, but that it is not clear whether glucocorticoids exert a large tonic inhibitory effect on lymphocyte function in humans.

Summary

Interactions between catecholamines, principally NE and E,

and the immune system appear to be complex and may seem contradictory since catecholamines appear to be simultaneously enhancing and inhibiting aspects of immune status. On the one hand, acute catecholamine stimulation appears to enhance immunity by augmenting the migration of white blood cells into the peripheral blood, particularly CD8⁺ and NK cells. On the other hand catecholamines are thought to suppress lymphocyte proliferation to mitogenic challenge. Although, not universally agreed upon, CD8⁺ cells are thought to include two subclasses, cytotoxic T cells which combat virally infected cells and tumors, and suppressor cells which may suppress immune activity.

NE, ISO and E infusions appear to stimulate an increase in numbers of lymphocytes and granulocytes. These effects are blocked with β -receptor antagonists, suggesting that β -receptors are on lymphoid tissue which house the cells. Psychological stress and exercise, which induce elevations in NE and E, are associated with increases in circulating immune cells, particularly CD8⁺ and NK cells. Proliferation of lymphocytes to mitogens is reduced following elevations in β -receptor agonists associated with psychological

stressor exposure, exercise, and injections of E or ISO. Further, blocking the effects of E with propranolol mitigate effects of E on lymphocyte proliferation.

B-adrenoreceptor number appears to be greater on circulating lymphocytes following infusions of NE, E and ISO and after mental arithmetic and exercise. Increase in β -receptor density is associated with elevations in intracellular cAMP accumulation. With enhanced cAMP synthesis there is a decrease in the ability of lymphocytes to proliferate to mitogens, suggesting one mechanism for stress-related reductions in lymphoproliferation.

Biphasic responses in receptor density and cAMP content may represent an adaptive mechanism. Initially, in response to elevated E levels during stress there may be an increase in lymphocytes with increased β -receptor density. This would facilitate binding of E and an inhibitory effect on immune function, possibly to avoid responses such as inflammation or fever while the stressor is being confronted. With time there may be a decrease in β -adrenoreceptors in order to reduce lymphocyte binding to

catecholamines so that suppression of immune function can be restored to resting states. Whether the diminution of β -adrenoreceptors is due to sequestering in the lymphocytes or to a redistribution of lymphocytes in circulation with fewer β -receptors on them remains to be determined. This question is important, since the combination of elevated lymphocyte numbers and an increase in β -receptors sequestered into the cell (thus, increasing cell function by reducing the cell's contact with catecholamines), overall immune function may be enhanced .

Rationale and Specific Aims

The specific aims of the study were as follows:

Aim I. To examine whether there is a change in surface and total β -adrenoreceptor numbers over the course of the session.

Aim II. To determine whether changes in surface β -adrenoreceptor density are due to sequestering of β -adrenoreceptors within the cell. Sequestering of β -adrenoreceptors was measured by determining if there is an increase in internal β -adrenoreceptors and a concomitant decrease in surface β -adrenoreceptors.

Aim III. To examine the time course of lymphoproliferative activity during mitogen exposure.

Aim IV. To examine whether altered lymphocyte proliferation responses to mitogens reflect changes in surface or total β -receptor numbers.

Subjects in the experimental group were asked to prepare and then give a speech. A baseline blood sample was taken in the beginning of the session. Blood was drawn again, in between the speech preparation and the actual speech, 5 minutes after the task, and 30

minutes after the task. Blood was examined for β -adrenoreceptor density (surface and total) and lymphocyte proliferation to mitogens.

Specific Aim 1.

- rationale: Prior research has described increases in β -adrenoreceptor number due to acute infusions of catecholamines. This change in receptors is associated with increases in intracellular cAMP which has been associated with decreased lymphocyte function (Carlson, Brooks & Roszman, 1989). It is not clear whether effects on the immune system from infusions of exogenous catecholamines reproduce effects that occur during psychological stressor exposure, even if they were infused at physiologic levels. Many hormonal changes occur during stress as do neurological and somatic changes. An objective of the present study was to determine whether acute psychological stress is related to elevations in β -adrenoreceptors.

Prior research also suggests that β -agonist exposure is associated with biphasic receptor response, an initial increase in β -

receptor density (Van Tits et al, 1990; Graafsma et al, 1985), followed by a decrease in β -receptor density (resulting in desensitization of lymphocytes to β -agonists) (Maisel et al, 1989). Decreases in some functional immune measures appear to occur as fast as 5 minutes after stressor exposure (Delehanty et al, 1994) which may be the result of immediate increases in β -adrenoreceptor density (increasing the sensitivity of cells to the inhibitory effects of elevated E levels). However, the time course for changes in β -adrenoreceptor density during and after psychological stressor exposure have not been examined. Thus, another objective of the study was to examine the time course involved in immune cell β -adrenoreceptor density before, during and following a laboratory stressor.

Specific Aim 2.

- rationale: Recently, studies have suggested that with prolonged exposure to β -agonists, surface β -receptors are sequestered into the cell. If receptors are withdrawn into the cell

and this inactivates them (E and NE cannot stimulate the receptors), this may represent an increase in immune function. Whether β -receptors are sequestered following stressor exposure has not been examined. An additional aim of the proposed study was to examine changes in β -receptor density on lymphocytes due to stress induced sequestering of β -receptors into the lymphocytes.

Specific Aim 3:

-rationale: Prior studies have found that increased catecholamines due to infusions of E (eg. Crary et al, 1983), exercise (eg. Murray et al, 1992) and psychological stressors (eg. Manuck et al, 1991) are associated with decreases in lymphoproliferation to mitogens. Few studies have examined the time course of these immune changes, before, during and after psychological stressor exposure. Drawing blood over the four time points in this study may help in determining a more definitive time frame of immune system changes.

Specific Aims 4.

- rationale: Previous studies have observed increases in lymphocyte β -adrenoreceptors in response to infusions of catecholamines (Korichnieva et al, 1990; Van Tits et al, 1991), exercise (Brodde et al, 1992; Middeke et al, 1992) and a psychologically stressful mental arithmetic task (Graafsma et al, 1987). However, lymphocyte function was not measured, and the extent to which the increase in receptors contributed to changes in immune function was not evaluated.

In the present study, in order to determine whether changes in β -adrenoreceptors were related to lymphoproliferative changes to mitogens over time, blood was drawn for β -adrenoreceptors and immune function at T1 the beginning of the session (baseline), T2 during the task (between speech preparation and speech presentation) in order to see immediate effects, T3 (five minutes after the task) to see if immediate changes in receptors were related to decreased immune function, T4 (thirty minutes after the task) to see if receptor density and lymphocyte function returned to baseline over time. The primary objective of the proposed study was

to determine whether changes in β -receptor density associated with psychological stress predicts changes in lymphocyte proliferation.

It may be adaptive for a large number of cells to be released from lymphoid tissue during and shortly after a stressful situation, however it may be beneficial to have these cells “turned off” (possibly with the combination of greater β -receptor density and elevated catecholamine levels) initially so that the organism does not have an immediate, large immune response which may slow down the organisms ability to respond to a stressful stimulus. During an immune response to a pathogen, immune cells secrete cytokines causing fever and malaise, which may not be conducive for running, fighting and decision making during a stressful situation (eg. fire, earthquake). Once the stressor has been removed and the effects of an immune response (feeling ill) are not as crucial, immunity may again return to an active state to fight against infections which may be a consequence of the stressful situation (cuts, burns etc.). Finding that immune activity recovers fairly quickly and is even enhanced shortly after stressor termination may suggest that immune responses to acute stressors are adaptive.

This was measured by adding a hydrophobic ligand, which binds to the receptors on the cell's surface along with the radioligand which binds throughout the cell to determine nonspecific binding.

Nonspecific binding was subtracted from total binding in order to determine specific binding on the lymphocyte cell surface.

A secondary goal was to investigate the time line of both β -receptor density and lymphocyte activity in order to determine if acute stress is associated with a biphasic response characterized by initial inhibition and a later enhancement or recovery of lymphocyte function and an initial increase and a later decrease in β -receptor number .

Hypotheses

The hypotheses of the study are as follows:

I. It is hypothesized that between speech preparation and before the actual speech (T2) there would be an increase in lymphocyte surface and whole cell β -adrenoreceptors. This would occur in response to anticipatory stress during the speech preparation. Ligands used to determine surface and whole β -adrenoreceptor density were 125 Iodopindolol (125 IPIND), CGP-12177, and propranolol, since each of these ligands bind to β -adrenoreceptors. 125 IPIND can bind to β -adrenoreceptors throughout the cell since it is hydrophobic and can therefore permeate the cell membrane and bind within, as well as, outside the cell. Also, it is radioactive and gamma emissions from the isotope can be measured with a gamma counter. CGP-12177 is an experimental drug which is hydrophilic and therefore binds only to surface β -adrenoreceptors, since it cannot enter into the lymphocyte through the cell membrane (which is hydrophobic). Propranolol is hydrophobic and can bind to β -adrenoreceptors throughout the whole cell.

Surface β -adrenoreceptor density was assessed by measuring gamma emissions from 2 million cells bound with $^{125}\text{IPIND}$ and CGP-12177, subtracted from gamma emissions from 2 million additional cells who were bound with $^{125}\text{IPIND}$ alone. Whole cell β -adrenoreceptor density was measured by subtracting gamma emissions from 2 million cells bound with propranolol and $^{125}\text{IPIND}$, from gamma emissions from 2 million additional cells who were bound with $^{125}\text{IPIND}$ alone (for β -adrenoreceptor binding protocol, see appendix II).

II. It is hypothesized that following speech preparation and before giving the speech (T2) there would be a decline in mitogen stimulated lymphocyte proliferation. A lymphocyte proliferation assay was used to measure this (see appendix II); reduced proliferation of stimulated cultured cells were hypothesized to represent an immediate suppression of immune activity in response to anticipatory stress during the speech preparation.

III. It is hypothesized that in response to giving a speech, mitogenic responses to Con A and PWM would be reduced shortly after the task. Others (eg. Delehanty et al, 1994) have found

changes in immune activity 5 minutes following a challenging/stressful task. Thus, it was hypothesized that there would be a significant decrease in mitogen-stimulated lymphocyte proliferation five minutes after the speech task (T3) (see appendix II).

IV. It is hypothesized that surface β -adrenoreceptors would begin to decrease and internal receptors would increase within 5 minutes following the stressor (T3), representing an initial sequestering of surface β -receptor numbers. This hypothesis was generated to explain how immune function is initially "turned off" (with increased surface β -receptors, immune cells would be stimulated by NE and E and thus more influenced by the immunosuppressive effects of catecholamines) and then gradually turned back on (with a decrease or sequestering of surface receptors). This was measured by comparing surface β -receptor density to whole cell β -receptor density. The methods of measuring surface and whole immune cell β -receptor numbers was the same as described in hypothesis I.

V. It is hypothesized that by 30 minutes after stressor

exposure (T4) lymphocyte function would be “turned back on” by reducing surface β -adrenoreceptors and therefore reducing the influence of catecholamines on the cells. By 30 minutes after termination of the stressor it is hypothesized that surface β -adrenoreceptors would be reduced significantly and the β -adrenoreceptors within the lymphocytes (ie. sequestered) would be significantly increased.

VI. It is hypothesized that lymphocyte mitogenic responses would return to normal after a period of time without stressor exposure. Therefore, it is hypothesized that 30 minutes after the speech (T4) lymphocyte proliferation would return to baseline.

VII. It is hypothesized that NE and E influence lymphocyte ability to respond to a mitogen by increasing β -adrenoreceptor density. Catecholamines circulating in the blood stream would more likely have contact with surface receptors on lymphocytes than receptors within the cell. Thus, catecholamines would be more likely to influence lymphocyte activity via surface receptors than internal receptors. It was therefore hypothesized that surface but not whole

lymphocyte β -receptor density would predict lymphocyte proliferation.

Methods

Subjects: Subjects were 40 male volunteers, recruited from among people responding to advertisements in the Washington Post, the Gaithersburg and Bethesda Gazettes and the NIH record; they ranged in age from 18 to 45. Male subjects were recruited in order to examine immune changes without influences of menstrual cycle hormonal changes. Young adults (18 - 45 years old) were examined in order to restrict the effects of age on endocrine and immune measures. Based on previous studies of acute stress and immune function and on power analyses it was determined that a total of 40 subjects would be needed to achieve adequate power. To ascertain the number of subjects necessary to detect an effect, formulas from Cohen (1988) were used. Initially a formula was chosen for an ANOVA because group (stress and no stress groups) differences in immune function were of primary importance. The effect size of .53 was computed using data from a study done by Zakowski, McAllister, Deal & Baum (1992), who also examined group (stress and no stress groups) differences in immune function. Alpha was set at 0.05.

Based on these criteria it was determined that 12 subjects were needed for each of the two groups. A power level of 0.80 was judged to be adequate in detecting potential effects. An additional power analysis for an ANOVA using 8 groups (4 time points and one experimental and one control group) was performed with an $\alpha=.05$ and an effect size of 0.40 (chosen since it is considered a moderate effect size) and a power level of 0.80 was performed revealing that 8 subjects were needed per group totaling 64 subjects. Because not all time points were expected to be of equal influence in the study, a compromise was made and 40 subjects, instead of the 24 suggested by the Zakowski et al. (1992) study and instead of the 64 suggested by the power analysis using 8 groups, were deemed necessary.

Procedures: Subjects arrived at the laboratory between 9:00 and 10:00 am. Upon completion of the consent form (see appendix III) subjects rested for 15 minutes before having a blood pressure cuff placed on their dominant arm. Five baseline heart rate and blood pressure measurements were taken at two minute intervals. A

catheter was then inserted in the non-dominant arm and subjects were asked to relax for an additional 20 minutes and fill out questionnaires on demographic information, as well as prestress psychosocial measures. Following the rest period, twenty mls of blood were drawn for a baseline measure of β -adrenoreceptor density (surface and total) and lymphoproliferation to Con A and PWM. Then subjects in the experimental group prepared a speech for 5 minutes, after which there was a second 20 ml blood draw. Subjects in the control group were engaged in a task of reading simple words such as cat, go, ball to themselves for five minutes (following which there was a 20 ml blood draw) and then reading the words out loud for an additional 5 minutes to control for speaking during the session. Blood was drawn 5 and 30 minutes after the speech. Heart rate and blood pressures were measured before, during and after the tasks throughout the session. Following performance of the tasks, questionnaires were again administered.

Tasks: The experimental group was given directions for giving the speech. Subjects had 5 minutes to prepare and 5 minutes to give the

speech. During the speech subjects were video-recorded. The directions were as follows:

The task that I would like you to do today is a speech task. This involves preparing a speech telling something about yourself, and then delivering the speech while you are being recorded on video.

I would like you to speak about a personality characteristic of yours which you like the least.

What is it?

What do you dislike about it?

In which ways would you change it if you could?

How do you deal with having this least desirable characteristic?

You have five minutes to prepare the speech in your head without the use of notes, and five minutes to give the speech. You may begin preparation. (set timer)

Subjects in the control group were asked to read to themselves a passage from a magazine selected for simple language and non-emotion eliciting content and then were asked to read it out loud. The directions were as follows:

In order to examine the effects of speech on physiologic measures such as heart rate, blood pressure and immune measures. I would like you to read these words to yourself and in 5 minutes I will ask you to read them out loud. You are not

being evaluated on your performance. Once again we are simply looking at physiologic measures of how your body responds to speech. Do you understand? Good. You may begin reading the words to yourself (set timer).

(5 minutes)

You may now begin reading the words out loud.

Physiological measures: A blood pressure cuff from a Spacelabs blood pressure monitor was placed on the subject's dominant arm at the beginning of the session. Baseline heart rate and blood pressure were measured 5 times in two-minute intervals during the last 15 minutes of the second pretask rest period. During the task, blood pressure and heart rate were measured 5 times at two minute intervals. Following the task blood pressure was measured at 5 minute intervals for the remainder of the session.

Blood draws: A butterfly catheter which was flushed with heparinized saline solution was inserted into the antecubital vein of the nondominant arm and remained there for the remainder of the session. After a 20 minute rest period, twenty mls of blood were drawn for biochemical measures (β -adrenoreceptor density and

lymphoproliferation). Blood was again drawn (20 mls) after the speech preparation task or reading a list of simple words in order to examine changes occurring during the stressor. Additional blood draws of 20 mls/draw were made 5 minutes and 30 minutes post task (speech/ reading simple words out loud) for a total of 80 mls per session.

Biochemical measures/ Assays: For all subjects, β -adrenoreceptor density (both surface and total), and proliferation of lymphocytes to mitogens were measured. Iodopindalol binding was used to examine β -adrenoreceptors, with propranolol and CGP-12177 as competitive ligands (propranolol to measure total receptors throughout the cell and CGP-12177 to measure surface receptors). The concentration of ^{125}I PIND used during the study to measure changes in receptor density was 125 Pm since this amount was used in a study by Maisel and Motulsky (1987) to bind 80%-90% of the β -receptors (see appendix I). A lymphocyte proliferation assay measured cell proliferation, with tritiated thymidine as the radiolabel and Con A and PWM as the mitogens (see appendix II).

Self report measures (see appendix III for questionnaires) : Self report measures were primarily administered for two reasons: to screen for subject differences that could unintentionally influence dependent measures, and to check that the manipulation in the study worked. At the end of the session a public speaking anxiety questionnaire was given to subjects to determine how stressful subjects viewed speaking in public. This 30 question true/false questionnaire was administered to the speech group to measure for individual differences in fear of public speaking. A stressor evaluation form was given to subjects in both groups after the tasks. The questionnaire was a Likert-style index with scales ranging from 1 (not at all) to 5 (extremely) for how anxious, tense, stressed, angry, interested and aroused the subjects felt during the task. This questionnaire was administered to check whether the tasks in the two groups differed in evoking self reports of stress. In order to determine whether there were demographic differences between the groups that could affect physiologic or biochemical measures a background questionnaire was administered to subjects.

Sociodemographic characteristics including age, income, employment, marital status and educational level were used to determine if there were group differences regarding these factors. A food checklist was completed to check whether foods were eaten in the previous 12 hours which could affect immune status. Subjects were told not to drink any caffeinated beverages or eat any fatty foods for the 12 hours before the session begins. The SCL-90R (Derogatis, 1977), which is a symptom checklist, was administered in order to screen for psychopathologic tendencies. It has subscales measuring items such as depression, and anxiety. A schedule of recent events (SRE) questionnaire (Holmes & Rahe, 1967) was administered to discover whether there were major stressful events in the subjects' lives which could have influenced dependent measures in the study. The questionnaire is composed of a list of 55 possible events that may have occurred in the past 0-6 months, 6-12 months, 12-18 months and 18-24 months. Adjustment scores were also recorded measuring from 0 (not at all) to 100 (greatly) how much the events affected the subject .

Statistical Analysis

Comparisons of variables among the experimental and control groups were performed by repeated measures analysis of covariance (ANCOVA), repeated measures analysis of variance (ANOVA), and multiple regression. Each ANCOVA was performed two times, once each for levels and change scores for variables such as blood pressure or lymphocyte proliferation. Oneway ANOVAs were used to determine if there were baseline differences between groups. Where baseline differences were found to be significant or marginally significant, baseline measures were used as covariates.

Results of repeated measures ANCOVAs for levels of blood pressure, heart rate and immune response to mitogen challenge were identical in the analyses using change scores. However, there were advantages to using both procedures. For example, when repeated measures ANCOVA mean levels were plotted, group X time interactions were easier to visualize, whereas picturing changes from baseline was easier with change scores. Also, post-hoc procedures measuring changes from baseline were possible only with change scores, since baseline was covaried out of the repeated

measures ANCOVA using levels. Post-hoc comparisons, directed at determining differences between groups and between responses within a group, were done using Tukey procedures, following methods outlined by Stevens (1992).

For hypotheses I, IV and V involving β -adrenoreceptor density across time, repeated measures ANCOVAs were performed on levels of β -adrenoreceptors considering one within-subject factor (Time) and one between subjects factor (group). Time had three levels; T2 (in between the speech preparation/reading to self, and giving the speech/reading aloud), T3 (5 minutes after the task), and T4 (recovery at thirty minutes after the task) (see Figure 1 for timeline). The between subjects factor group variable had two levels as well, experimental (speech) and control (reading). T1 (baseline) measures were used as covariates. Repeated measures ANCOVAs were also performed on change scores obtained by subtracting values at times 2, 3, and 4 from baseline at each time point, with one within subject factor (Time) with three levels T2 - T1, T3 - T1 and T4 - T1. There was again one between subjects factor (Group) with two levels, experimental (speech) and control

(reading).

Insert Figures 1

For hypotheses II, III and VI analyses were again performed with repeated measures ANCOVAs on levels of lymphoproliferation to mitogen, and changes in lymphoproliferation from baseline, considering the same between and within subject factors as were considered for β -adrenoreceptor density. T1 (baseline) measures were used as covariates. Post hoc comparisons (Tukey) were conducted to examine differences between groups and between responses within a group. For hypothesis VII multiple regression analyses were conducted in order to predict the percent of variance in lymphocyte proliferation accounted for by beta-adrenoreceptor density. Group was entered first in order to remove the influences of group differences, followed by SBP, β -adrenoreceptor density, and lymphoproliferation to mitogen.

An increase in sympathetic arousal (eg. evidenced by elevations in catecholamines, heart rate, and blood pressure) may be

related to changes in immune function. Some studies found that subjects with higher physiologic reactivity to a task had a less vigorous lymphoproliferative response to mitogens, compared with subjects with lower physiologic reactivity (eg. Manuck et al, 1991; Zakowski et al, 1992). However, other studies did not find this relationship (eg. Zakowski et al, 1994; Glaser et al, 1994). In order to examine whether physiologic reactivity was associated with lymphoproliferation in the present study, subjects in the experimental group were separated into high- and low- sympathetic reactors to the speech task by separating them into groups above and below the median for SBP, DBP, and HR. A repeated measures ANCOVA was then performed on levels of lymphoproliferation to mitogen, and changes in lymphoproliferation from baseline, considering the same within subject factors as were considered for β -adrenoreceptor density and lymphoproliferation to mitogen (without accounting for reactivity). However, in this case the between subject factor group variable had three levels, experimental group high-reactor, experimental group low-reactor, and control group. A repeated measures ANCOVA was performed for

Con A and PWM, for each physiologic measure, SBP, DBP and HR. T1 measures were used as covariates. Post hoc comparisons (Tukey) were conducted to examine differences between groups.

Finally, ANOVAs were used to evaluate self reports of stress, tension, anxiety, anger, interest and arousal in response to the tasks. Dependent measures evaluated in the study included proliferation of lymphocytes to ConA 5, ConA 10, PWM .01, PWM .05, surface β -adrenoreceptor density, β -adrenoreceptor density throughout the whole cell, internal β -adrenoreceptor density, systolic blood pressure, diastolic blood pressure, heart rate, anxiety, tension, stress, interest, anger, and arousal.

Results

Physiological Activation

Changes in heart rate and blood pressure were evaluated as an index of physiologic activation and sympathetic nervous system arousal during the laboratory session. Oneway ANOVAs revealed significant baseline differences between groups for diastolic blood pressure (DBP) $F(1,35)=5.34$, $p<.03$ and marginally significant baseline differences between groups for systolic blood pressure (SBP) $F(1,35)=3.09$, $p<.09$ and heart rate (HR) $F(1,35)=2.48$, $p=.12$ (SBP, DBP and HR baselines were higher in the control group than the experimental group). In these cases, baseline was used as a covariate, to remove the influences of differing baselines. For SBP, DBP and HR, results indicated significant time effects, $F(2,58)=10.11$, $p<.001$, $F(2,58)=12.22$, $p<.001$ and $F(2,58)=17.20$, $p<.001$ respectively, indicating higher blood pressure and heart rate levels during the tasks than at baseline and post-task time points. There were significant group X time interactions for SBP $F(2,58)=5.47$, $p<.007$ and DBP $F(2,58)=4.35$, $p=.017$, and a marginally significant group X time interaction for HR $F(2,58)=2.68$,

$p = .077$, with higher SBP, DBP and HR levels achieved by the experimental group during the speech compared to baseline and post task. Means for these analyses are presented in figures 2, 3, and 4. Post hoc analyses indicated significant increases in SBP and DBP from baseline during the speech in the experimental group, and significant group differences for SBP and DBP for subjects giving a speech versus those reading aloud. Change scores for these analyses are presented in figures 5, 6, and 7.

Insert Figures 2 - 7

Among subjects giving the speech, mean baseline-to-task changes in systolic and diastolic pressure were +10 and +11 mm Hg respectively and for HR the change was 11 bpm. Control subjects showed corresponding systolic and diastolic pressure increases of +2 and +3 mm Hg respectively and an increase of +1 bpm for HR (for means and standard deviations of physiologic activity see table 1).

insert Table 1

Immunologic Changes

Proliferation of lymphocytes when exposed to mitogens ConA and PWM was measured as indices of immunologic activity. To normalize the data, a square root transformation was performed on measures of mitogenic response to Con A and PWM. Repeated measures ANCOVAs revealed an absence of both a Group X Time X Con A Concentration (ie. 5 ug/mls and 10 ug/ml) interaction and a Group X Time X PWM Concentration (ie. .01 ug/ml and .05 ug/ml) interaction indicating that these relationships generalized across the two concentrations for each mitogen. Oneway ANOVAs revealed significant baseline differences between groups for Con A $F(1,35)=4.35$, $p<.05$ and marginally significant baseline differences between groups for PWM $F(1,35)=3.14$, $p=.08$, with higher baseline levels in the experimental group. Therefore, baseline was used as a covariate to remove the influences of differing baselines.

There were group X time interactions for

lymphoproliferative responses to Con A $F(2,60) = 3.86$, $p = .026$ and PWM $F(2,52) = 3.79$, $p < .03$. Means for these analyses are plotted in figures 8 & 9 (for means and standard deviations for Con A and PWM stimulated lymphocyte responses are listed in table 2). Post hoc comparisons using change scores revealed significant group differences in lymphoproliferative response to PWM at T3, with decreases in response from baseline in the experimental group and increases from baseline in the control group. These data suggest that stress associated with giving a speech reduced lymphoproliferation to PWM. Change scores for these analyses are presented in figures 10 & 11.

 Insert figures 8 - 11

 insert Table 2

Receptor Changes

β – adrenoreceptor density was measured on lymphocyte cell

surfaces, throughout the whole lymphocyte, and within the lymphocyte. Baseline values were not significantly different between groups so a repeated measures ANOVA was used without covarying for baseline. There was a significant group X time interaction for surface β -adrenoreceptor density $F(3,81)=2.91$, $p = .04$ and whole cell β -adrenoreceptor density $F(3,81)=2.28$, $p = .04$. No significant effects were found for internal receptors $F(3,81)=.99$, $p = .40$ or ratios of surface/internal β -adrenoreceptors $F(3,81)=1.27$, $p = .29$. Post hoc analyses revealed that the experimental group had significantly greater surface β -adrenoreceptor density at T2, T3 and T4 than did controls and showed significant increases in receptor density from baseline to T2 and T3. Control values at T2, T3, and T4 did not differ from baseline. Means for analyses of surface β -adrenoreceptors are plotted in figure 12.

Insert figure 12

Predictors of Immunologic Change

Multiple regression analyses were conducted to examine the extent to which physiological activity and surface β -adrenoreceptor density predicted lymphoproliferation to mitogens. Both experimental and control groups were included in the equations; analysis could be conducted separately in the experimental and control groups, but would result in greatly diminished degrees of freedom. As a result both groups were included to preserve degrees of freedom in the analyses. Group was entered into the equation first to remove the effects of group on lymphoproliferation, followed by changes in independent variables from baseline to T3, which were entered into the multiple regression equation as follows: SBP, β -adrenoreceptor density, and mitogen elicited response.

When SBP was entered into the equation, it accounted for 8 and 6% of the variance for proliferation of lymphocytes to Con A and PWM respectively. Although SBP did not significantly account for variance in immunologic activity, it was included in the equation because SBP, as a measure of sympathetic arousal may share variance with β -adrenoreceptor density for lymphoproliferation.

Catecholamines may be the common thread between the two measures since catecholamines stimulate adrenoreceptors on arteries and the heart (therefore affecting cardiovascular measures), and are also related to changes in β -adrenoreceptor density. Catecholamines, in turn have been observed to affect lymphoproliferation (Crary et al, 1983). Since catecholamines were not examined in this study, entry of SBP in the equation decreased the percentage of variance accounted for by surface β -adrenoreceptors, suggesting that SBP and β -adrenoreceptor density may share variance for lymphoproliferation. By entering SBP into the equation, its influences on β -adrenoreceptor density were removed, so that the effects of β -adrenoreceptor density on lymphoproliferation could be identified without shared influences of sympathetic arousal. When DBP and HR were entered into the equation independently, each accounted for less than 1% of the variance, and appeared to share less of the variance with β -adrenoreceptor density for lymphoproliferation. They were not included in the equation.

After removing group and SBP, β -adrenoreceptor changes from baseline to post speech/reading accounted for 14% of the variance for changes in proliferation of lymphocytes exposed *in vitro* to Con A $F(3,22)=4.14$, $p=.05$ (see figure 13) and accounted for 23% in response to PWM $F(3,22)=7.9$, $p=.01$ (see figure 14).

insert figures 13 & 14

Cardiovascular Reactivity and Lymphoproliferation

Repeated measures ANCOVAs were performed to examine whether differences in cardiovascular reactivity to a stressful task (speech task) influenced immune activity during the various phases of the study. T1 was used as a covariate to remove the influences of differing baselines between the control and experimental groups. Significant group X time interactions were found for lymphocyte responses to Con A, for high- and low- SBP reactors, and controls $F(4,52)=2.64$, $p=.04$, for high- and low- DBP reactors, and controls $F(4,52)=3.57$, $p=.01$, and for high- and low- HR reactors, and controls $F(4,52)=3.02$, $p<.03$. Significant group X time interactions

were also found for lymphocyte responses to PWM, for high- and low- SBP reactors, and controls $F(4,52)=3.4$, $p = .015$ and for high- and low- DBP reactors, and controls $F(4,52)=2.57$, $p = .05$. There was a marginal group X time interaction for lymphoproliferation to PWM for high- and low- HR reactors, and controls $F(4,52)=2.30$, $p < .07$. The significant group X time interactions found with these analyses, suggest group differences in lymphoproliferative responses to mitogens across the various time points being examined during the study.

Post hoc comparisons revealed that although a number of group X time interactions were found, these were primarily due to differences between the control group and the experimental group (high- and low- sympathetic reactors) across time, and may not be due to differences between high- and low- reactors. The control group differed significantly from high- and low- SBP and DBP reactors in lymphoproliferation to PWM at T3, where controls increased, and both high- and low- SBP and DBP reactors decreased in lymphoproliferation. The control group also had an increase in lymphoproliferation to PWM compared with low- HR reactors, who

decreased in lymphoproliferation from baseline at T3. At T4, the control group differed in lymphoproliferation to PWM from low- SBP reactors and high- HR reactors.

At T3, when heart rates and blood pressures changed the most during the study, there were no differences between high- and low- SBP, DBP and HR reactors for lymphoproliferation to Con A or PWM. However, group differences between high- and low- reactors were found at T2 (following speech preparation); high- DBP reactors had a decreased lymphocyte response to Con A from baseline , whereas low- DBP reactors had an increased lymphocyte response from baseline. Also, at T2 and T4 high- SBP reactors had a weaker lymphoproliferative response to PWM compared with low- SBP reactors . Finally, at T4 low- DBP reactors had a decreased lymphocyte response to PWM compared to high- DBP reactors, which had an increased response.

These data suggest a minimal relationship between high- and low- reactivity and lymphoproliferation, in the present study. The differences found were inconsistent across mitogens and across cardiovascular measures. For means and standard deviations of

lymphoproliferation to Con A and PWM in high- and low-sympathetic reactors, and controls see Table 3.

insert Table 3

Self Report

Measures of tension, anxiety, stress, anger, interest and arousal were taken immediately following the tasks as a manipulation check. Oneway ANOVAs revealed that there were significant group differences in tension, anxiety, and stress $F(1,33) = 33.3, p = .001$; $F(1,33) = 35.1, p = .001$; $F(1,33) = 15.8, p = .001$ respectively with the experimental group having significantly higher scores on these measures (*see figures 15,16 & 17*). There were no effects for interest, anger, or arousal.

insert figures 15, 16 & 17

DISCUSSION

The present study found that subjects giving a speech had increased lymphocyte β -adrenoreceptor density and decreased PWM induced lymphocyte proliferation compared to subjects reading simple words aloud. These effects appeared to be attributable to stress. Changes in lymphocyte β -adrenoreceptor density significantly predicted changes in lymphocyte proliferation to Con A and PWM, independent of group. This study was designed to examine one possible mechanism of stress-induced immune system decrements, namely the role of lymphocyte β -adrenoreceptor density on immune cell lymphoproliferation during and after acute stress. Alterations in β -adrenoreceptor density following speech preparation and presentation were examined to determine whether psychological stress induced β -adrenoreceptor density changes mediate changes in lymphocyte activity. Results provided partial support for this hypothesis, indicating that acute stress increased receptor density and this contributed to decreases in proliferation. The results from the present study are consistent with results of

previous studies. Prior studies have found reductions in lymphocyteresponses to mitogen stimulation (eg. Con A, PWM and PHA) during and following participation in stressful tasks (eg. Bachen et al, 1992). Infusions of E have also been related to decreased responses to PWM, PHA and Con A in humans (eg. Crary et al, 1983). In the present study, subjects in the experimental group exhibited a less vigorous lymphocyte response to PWM after the speech task, compared with control subjects who exhibited a small increase in proliferation after reading simple words aloud.

Also, since giving a speech has been observed to double E levels but not significantly increase NE (Dimsdale & Moss, 1980) the increase in surface β -adrenoreceptor density after speech preparation and after giving a speech in the present study is consistent with investigations of acute infusions of isoproterenol and E, which found increased β -adrenoreceptor density (Van Tits et al, 1990).

Although other studies have not looked at the relationship between β -adrenoreceptor density and lymphoproliferation,

increased β -adrenoreceptor density has been related to increased cAMP accumulation (Sutherland, Robison & Butcher, 1968; Carlson, Brooks & Roszman, 1989), which in turn is thought to inhibit lymphocyte function (Murray et al, 1993). This suggests that there is a connection between β -adrenoreceptor density and lymphocyte action. In the present study this was found to be true; changes in receptor density were predictive of lymphocyte activity. Surface β -adrenoreceptor density accounted for 14% of the variance for lymphocyte proliferation to Con A, and 23% for proliferation to PWM five minutes after the completion of the tasks (either giving a speech or reading aloud), independent of group.

Although, there were increases in heart rate and blood pressure following the speech task (compared to control subjects) it was surprising to find that they were not obviously related to lymphoproliferative responses following the speech task. Even after subjects in the experimental group were separated into groups of high- and low- reactors, physiological measures were still for the most part unrelated to immune measures. This is inconsistent with some studies but not with others. In one study, acute stress and the

magnitude of the associated cardiovascular reactions (heart rate, systolic and diastolic blood pressure) was related to reduced lymphocyte proliferation to mitogens (Zakowski et al, 1992).

However, in another study SBP, DBP, HR and catecholamine measures were combined in order to find a relationship between sympathetic arousal and lymphoproliferation (Manuck et al, 1991). Still others did not find a relationship between cardiovascular measures and lymphoproliferation (Glaser et al, 1994; Zakowski et al, 1984). In the present study and perhaps in other studies, by measuring heart rate and blood pressure during the task and then measuring mitogenic responses five minutes after the task, subtle correspondence between physiological activity and immunity may have been missed. Timing of measures of sympathetic nervous system arousal and immune activity may be important in this regard.

Some investigators have suggested that catecholamine-induced increases in β -adrenoreceptor density reflect subpopulations of cells released from lymphoid tissue with greater numbers of β -adrenoreceptors on them (Khan et al, 1986). Whether an eventual decrease in β -adrenoreceptors also reflects

subpopulations of lymphocytes being redeposited into lymphoid tissue after catecholamine levels return to normal has not yet been determined. It has been suggested that reductions in β -adrenoreceptor number are not due to recirculation of lymphocytes but to sequestering of receptors within the cell (DeBlasi et al, 1985). Thus, the present study sought to determine whether β -adrenoreceptors were sequestered over time, after the stressor had been removed. In order to measure this, surface/internal receptor ratios were determined. For sequestering to have occurred there would have been an increase in internal receptors over time after the stressful task and/or a concordant decrease in surface β -adrenoreceptors, thereby reducing the ratio. However, over the thirty minute post-stressor period there was no change in numbers of internal receptors, or in the ratio. Although not significant, internal receptors actually decreased over this time period. Therefore, the decrease in surface receptors following the stressor may be attributable to recirculation of receptors (this cannot be proven in this study) but do not seem to be related to sequestering.

Whether complete measurements of all β -adrenoreceptors within the lymphocytes were taken, can not be determined. It is possible that the ligands may not have completely entered into the lymphocytes since intact cells were used. A broken cell preparation may have revealed more accurately the number of β -adrenoreceptors throughout the lymphocytes. However, the act of breaking the cells (either by homogenizing or lysing) may damage the receptors and therefore cause other measurement problems. Measurements of whole lymphocyte β -adrenoreceptor density were collected in the study in order to determine whether whole lymphocyte β -adrenoreceptor density or surface β -adrenoreceptor density change following a stressful task. Whole lymphocyte measures of β -adrenoreceptor density probably do not differ greatly from surface β -adrenoreceptors, since internal receptors do not change significantly over time, and so the change that is occurring is due mainly to surface β -adrenoreceptor density.

An elevated lymphocyte response occurring post-stressor may be adaptive for an organism in need of additional immunologic

activity resulting from the stressful event (eg. to fight infections that might occur with cuts, burns etc.). In this study, the thirty minute post-stressor recovery period was examined for mitogenic responses that might have increased above baseline levels. Although there was an increase in proliferation above baseline at post-stressor 30 minutes, it was not significant. These results are difficult to interpret and do not necessarily suggest immunoenhancement.

In sum, hypotheses I, III, VI and VII were supported by this study. There was a significant increase in lymphocyte surface and total β -adrenoreceptor density in the experimental group after speech preparation compared to baseline (hypothesis I). Five minutes after the speech there was a significant decrease in lymphocyte proliferation to PWM but not to Con A compared to controls although, both mitogens produced significant group by time effects over the entire session (hypothesis III). By thirty minutes after the stressor lymphocyte proliferation returned to baseline (hypothesis VI). Surface β -adrenoreceptor density predicted a significant amount of the variance for lymphocyte proliferation to

both Con A and PWM (hypothesis VII).

Hypotheses II, IV and V were not supported by the present study. Although lymphocyte proliferation declined from baseline following speech preparation for both mitogens compared with controls, differences were not significant (hypothesis II). At five and thirty minutes after the speech task there was no change in internal receptor number suggesting that receptors were not being sequestered (hypotheses IV & V).

To further understand the mechanisms involved in immunosuppression during and following stress, further study is needed. For example, measuring total numbers of lymphocytes and subclasses of lymphocytes, and β -adrenoreceptor density on these subclasses during and following stress would provide new information concerning at least two things. First, this would indicate whether there are elevated numbers of lymphocytes following the speech task which remain elevated until thirty minutes after the stressor, when there is increased lymphocyte proliferation. This would suggest even more strongly an enhanced immune response after recovery, since a combination of elevated

numbers of immune cells combined with elevated mitogenic activity would most likely improve over-all immune function. Second, if subclasses of cells with greater numbers of β -adrenoreceptors on them increase following the speech and decline throughout the session (matching the increase and then slight decline in β -adrenoreceptor density found in this study), then it would be more apparent whether changes in β -adrenoreceptors are due to recirculation of cells. This could be important for further understanding how changes in β -adrenoreceptor density occur, which may play a role in the modulation of immune activity.

Measuring E and NE would also clarify the mechanisms involved, so that a direct comparison between β -adrenoreceptors and these hormones could be made. This would help tell a more complete story of immunoregulation by catecholamines. Although, heart rate and blood pressure were found to be related to lymphoproliferative differences in one study (eg. Zakowski et al, 1992), others have required measures of catecholamines combined with HR and blood pressure for this relationship to be realized (eg.

Manuck et al, 1991) or have not found such a relationship (Glaser et al, 1994).

In addition, it may be of interest to see how different tasks affect β -adrenoreceptor density and immune system responses.

Levels of NE and E appear to be task dependent (eg. Ward et al, 1983; Dimsdale & Moss, 1980) and these differences in tasks may differentially affect β -adrenoreceptor numbers and immune function. Physical exercise is associated with increased NE, whereas psychological tasks appear to be more related to increased E (Ward et al, 1983). Since E compared with NE has a 10-30 time greater affinity for β_2 -adrenoreceptors which are the dominant β -adrenoreceptor on lymphocytes, task effects may differ for β -adrenoreceptor density and immune function between physical and psychological tasks (Barns, 1981; Brodde, 1986). It would be of interest to determine if different mechanisms are involved in immune system changes depending on the types of tasks.

Proliferation of lymphocytes is only one measure of immune function; other segments of the immune system also need to be

addressed. For example, NK cells have been found to have even greater numbers of β -adrenoreceptors on them than T cells (Khan et al, 1986). However, the influence of catecholamines on NK cells resulting from psychological stressors has not been examined. NK cells are involved in surveillance of cancer cells and are important for health considerations (Riley, 1981). It would be of interest to do a similar study examining β -adrenoreceptors on NK cells and their relationship to NK activity. In addition, NK activity has been observed to increase during acute stressor exposure (Delehanty et al, in press) but to decrease during chronic stress (Kiecolt-Glaser et al, 1987). It would be of interest to see whether β -adrenoreceptor density is involved in regulation of these cells for both acute and chronic stress.

Different immune cell types may respond to different types of immunoregulators. For example, T and B lymphocytes have fewer receptors for glucocorticoids on them than other cells such as macrophages (Katz et al, 1985). It is possible that macrophages are better regulated by cortisol than catecholamines, just as T and B lymphocytes may be better regulated by catecholamines.

Differential regulation of the various immune cell types needs to be examined.

Other hormones should also be examined for their influences on immunity (eg. growth hormone, ACTH, prolactin) . Since β -adrenoreceptors only accounted for between 14 and 23% of the variance for changes in immune function following stressor administration, other variables (such as various hormones) may also be involved in immune system regulation which have not been examined.

Identifying mechanisms involved in immunosuppression may be important for future interventions. By knowing more precisely the mechanisms by which immune function is altered, stress techniques can be developed to specifically address these mediating factors. Few intervention studies have been performed to block the effects of stress on immune system changes. Those that have, either did not look at the physiological mechanisms involved in the immune system changes, or have not properly controlled for confounding variables. One study found that practicing Transcendental Meditation was related to decreased numbers of functional β -adrenoreceptors

on lymphocytes (Mills et al, 1990). A definition of “functional” β -adrenoreceptors was not given. Also, the frequency of meditation was not measured, nor did they examine whether meditation reduced the effects of stress on β -adrenoreceptor density. Finally, functional immune measures were not assessed to determine if meditation induced β -adrenoreceptor changes were related immune activity.

In another study, relaxation training was associated with increased immune system activity in the elderly (Kiecolt-Glaser et al, 1988). Also, medical students who regularly practiced relaxation techniques had better immune system function following exams than those who did not practice relaxation as frequently (Kiecolt-Glaser et al, 1989). However, the mechanisms involved in relaxation induced immune system changes were not examined.

SUMMARY AND CONCLUSIONS

This study was designed to examine the role of stress induced changes in β -adrenoreceptor density on lymphoproliferation

to mitogens. It was found that giving a speech was associated with increased β -adrenoreceptor density, and reduced PWM-stimulated lymphocyte proliferation. Changes in β -adrenoreceptor density predicted changes in lymphocyte proliferation to both PWM and Con A.

A relationship between changes in β -adrenoreceptor density and immune function was suspected, but had not been proven. The present study found a significant relationship between the two measures suggesting such a link. However, adreno-modulation of lymphocyte activity via β -adrenoreceptor stimulation is not the sole regulator of lymphocyte activity, since a large amount of the variance in lymphocyte activity is not accounted for by β -adrenoreceptor changes. More research is needed to discover additional mechanisms involved. Recognizing mechanisms involved in immunosuppression associated with stress may be important for future interventions.

Figure 1: Time-line

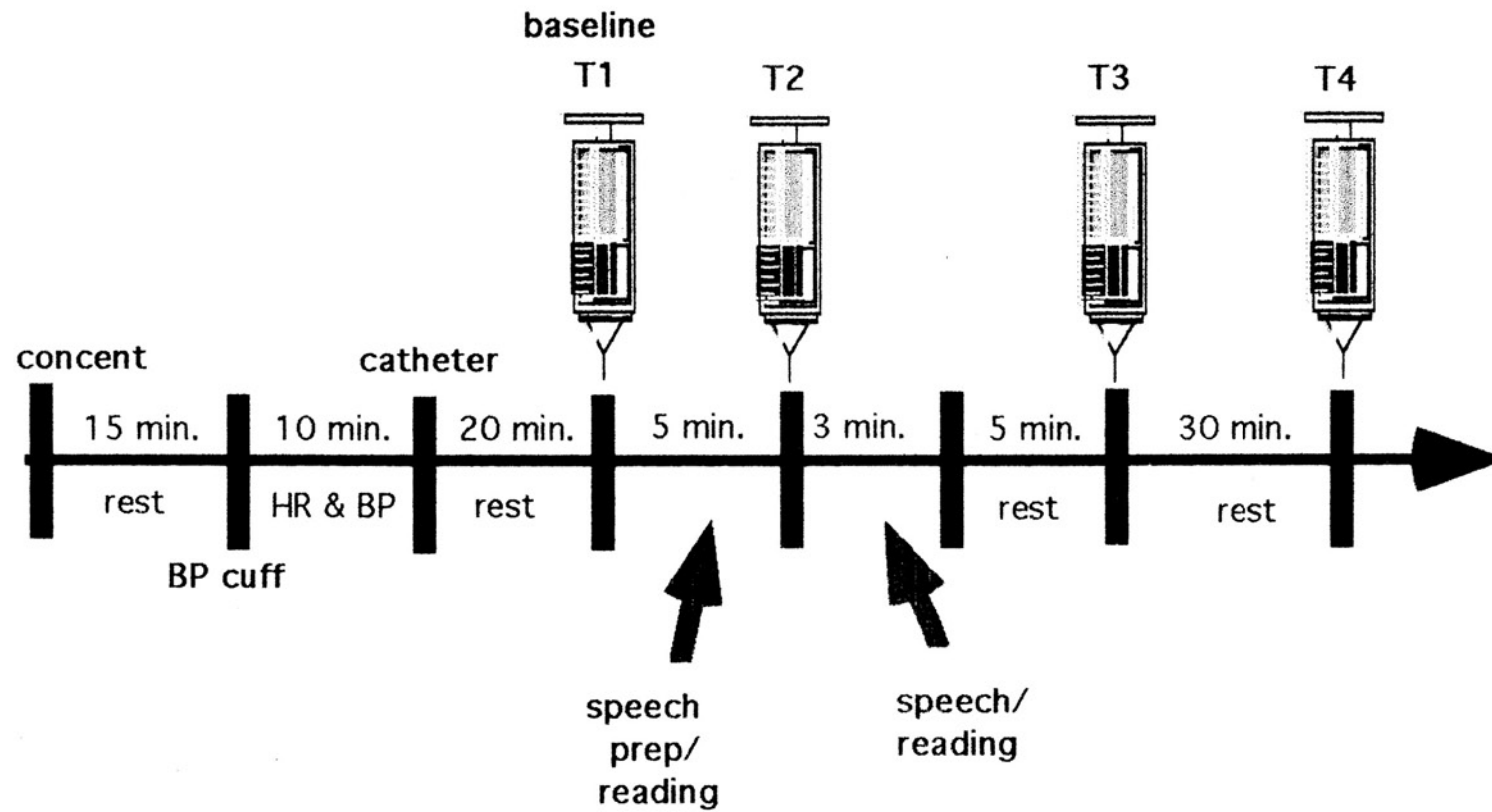


Figure 2:
Systolic Blood Pressure

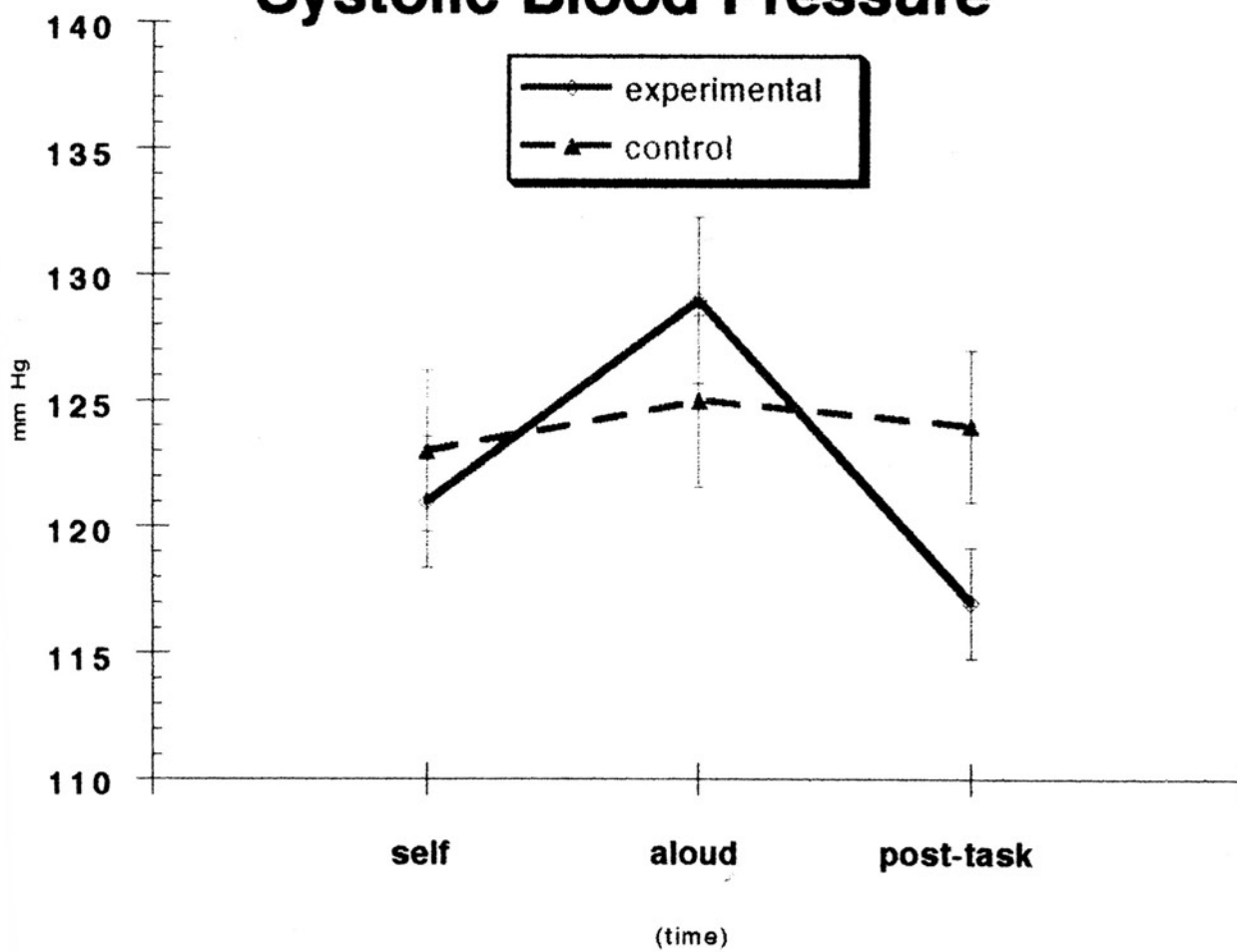


Figure 3:
Diastolic Blood Pressure

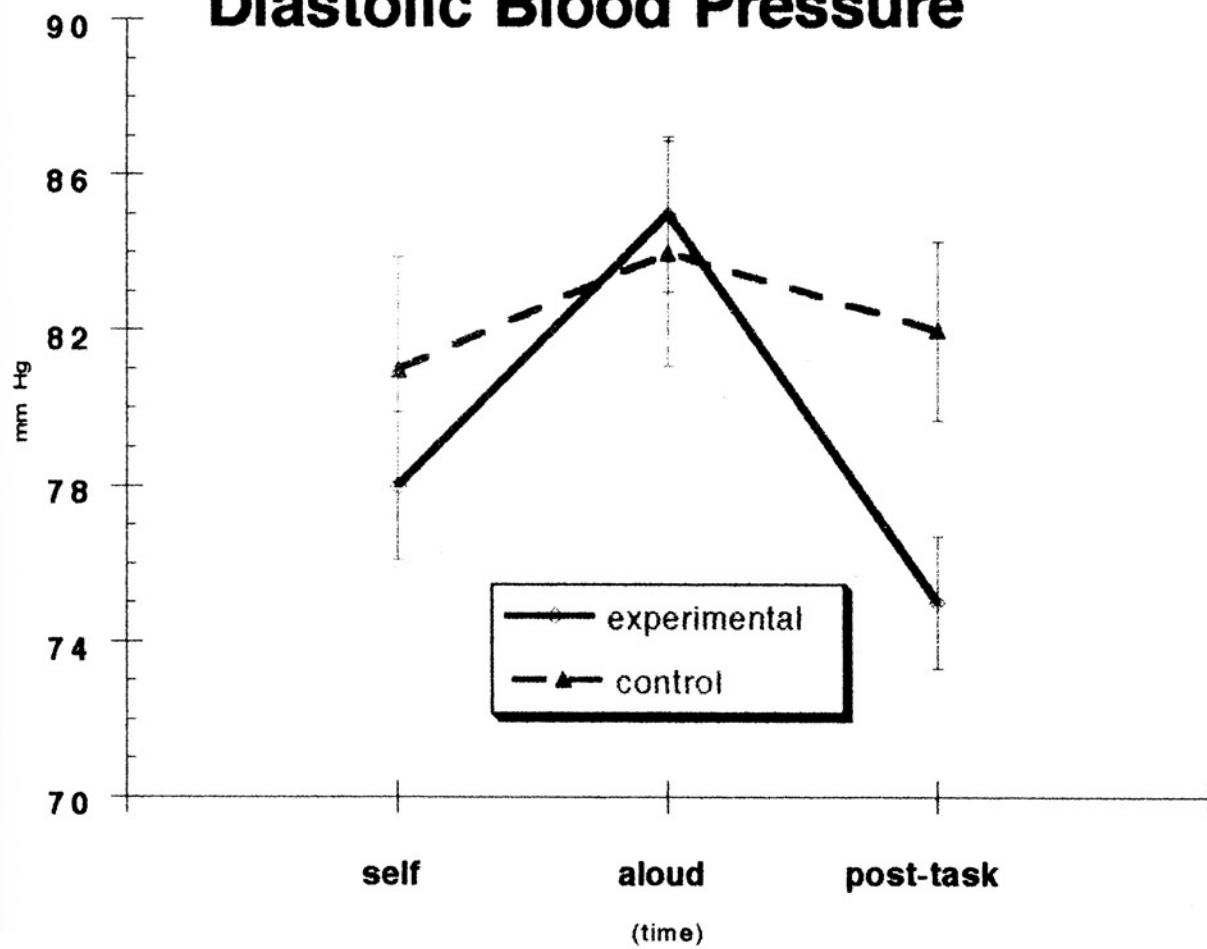


Figure 4: Heart Rate

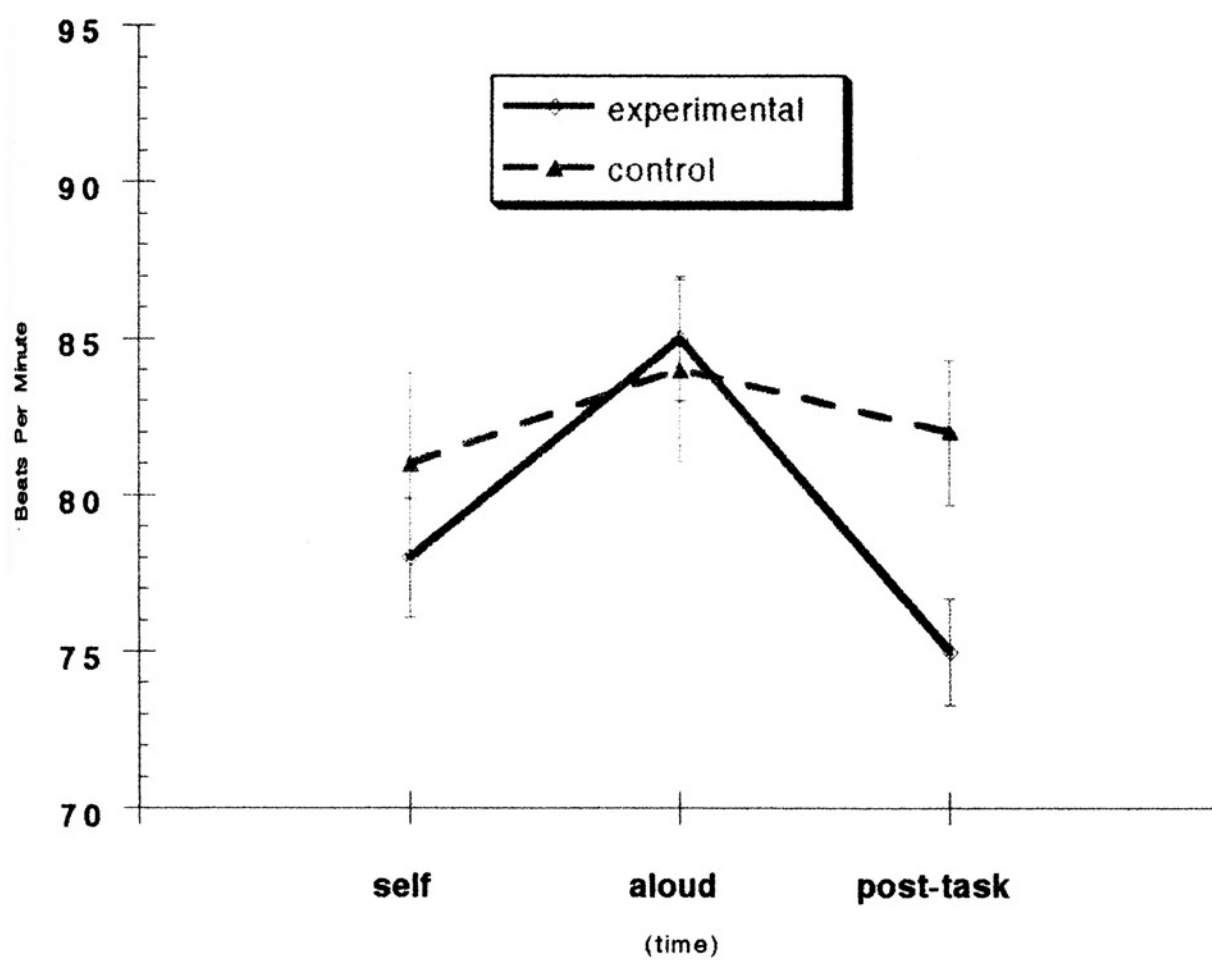
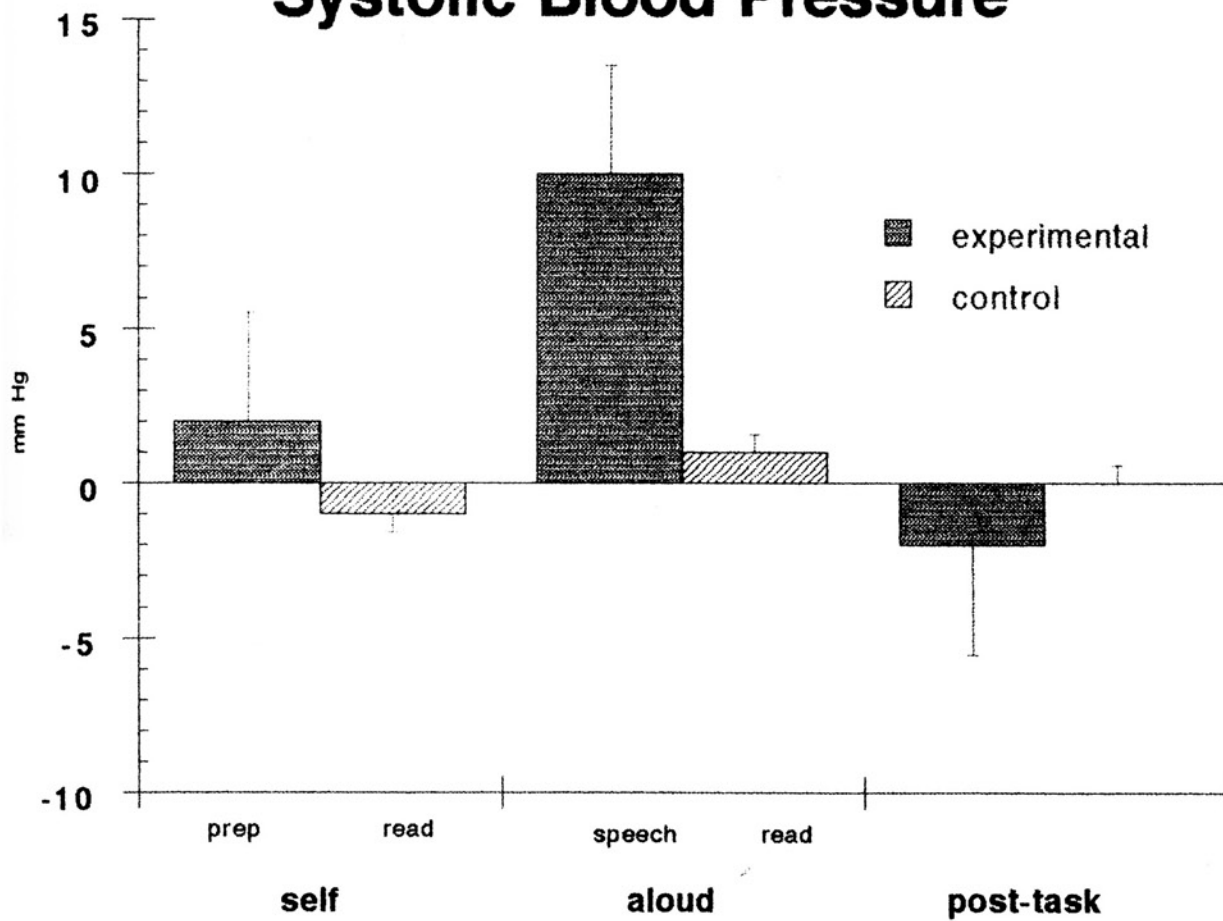


Figure 5:
Systolic Blood Pressure



**Figure 6:
Diastolic Blood Pressure**

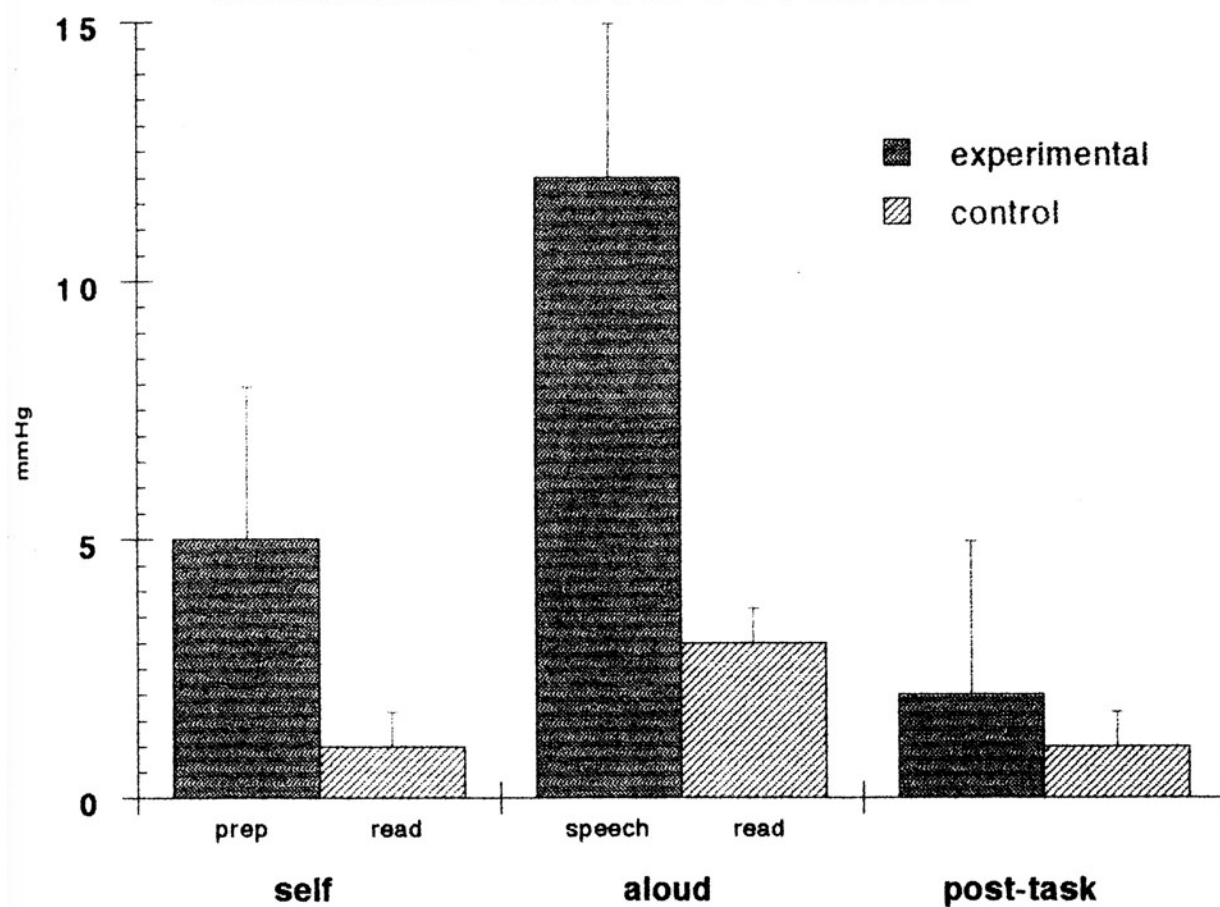


Figure 7: Heart Rate

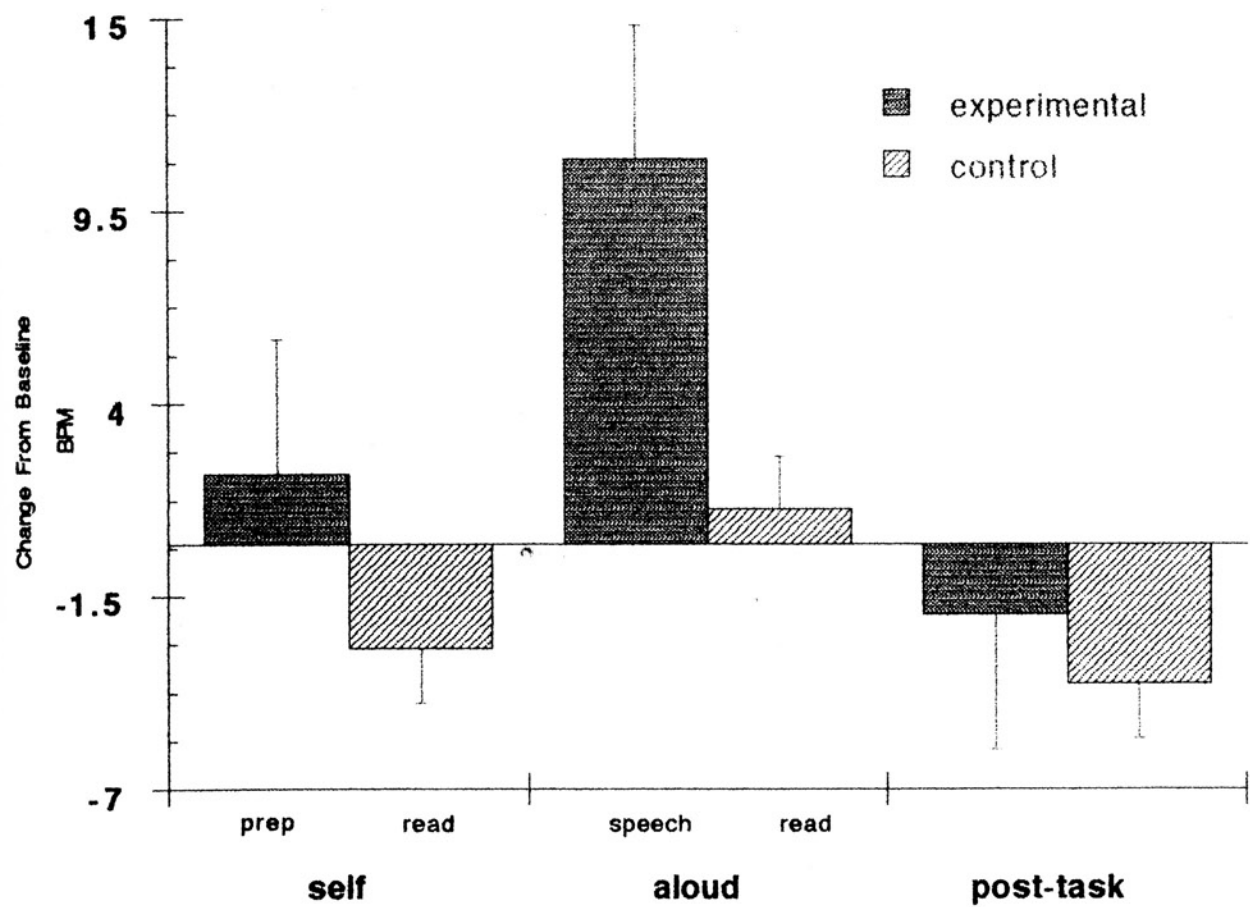
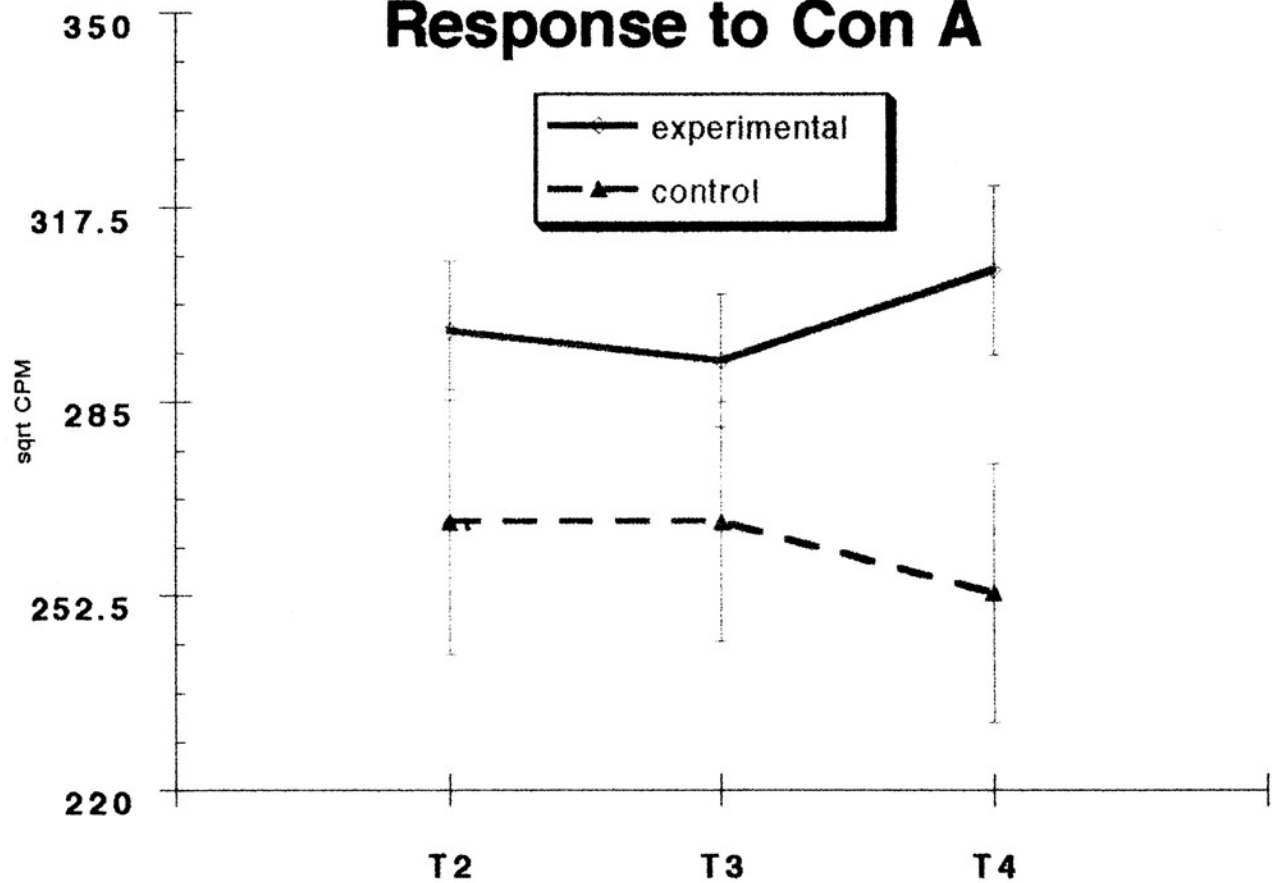
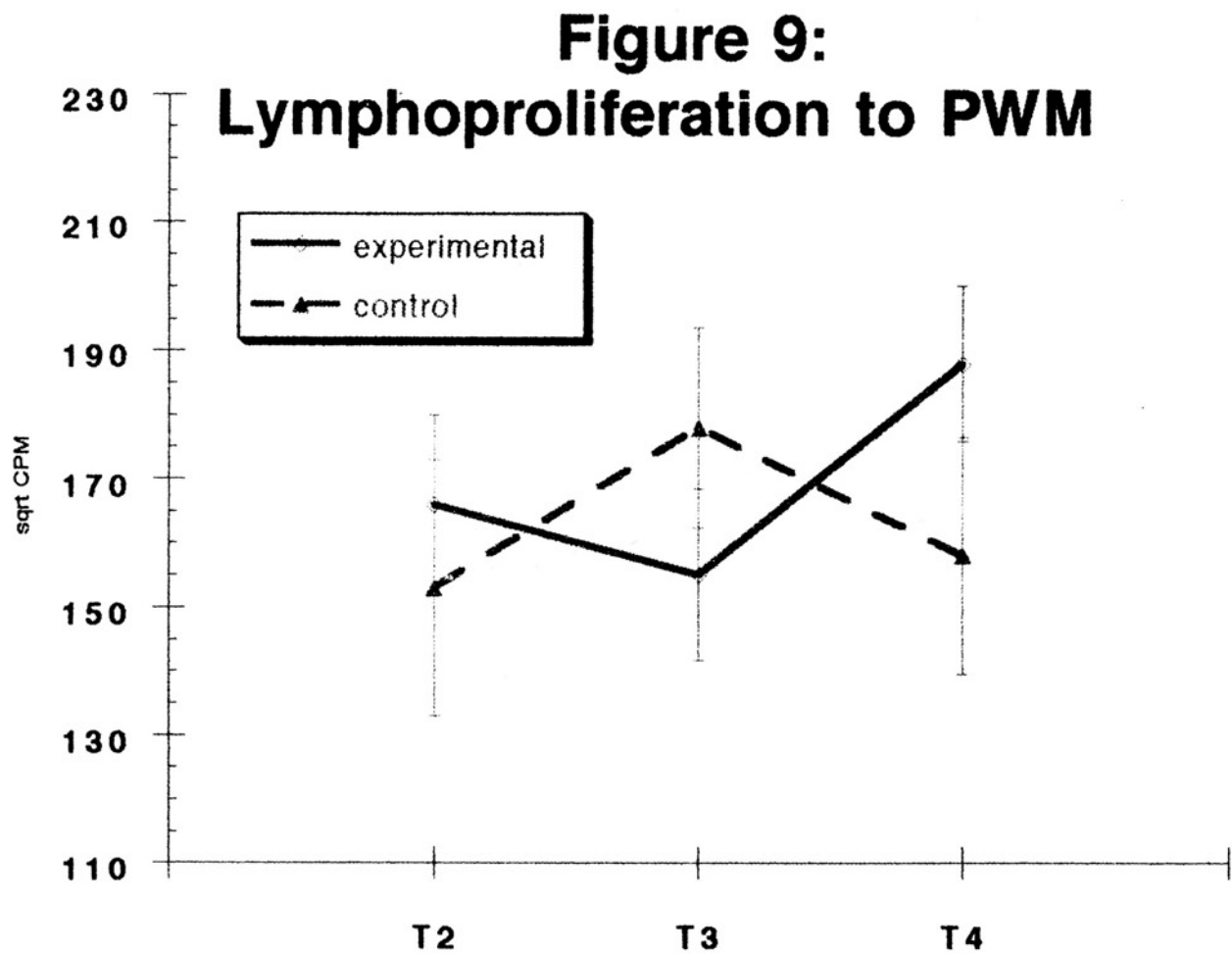


Figure 8: Lymphoproliferative Response to Con A

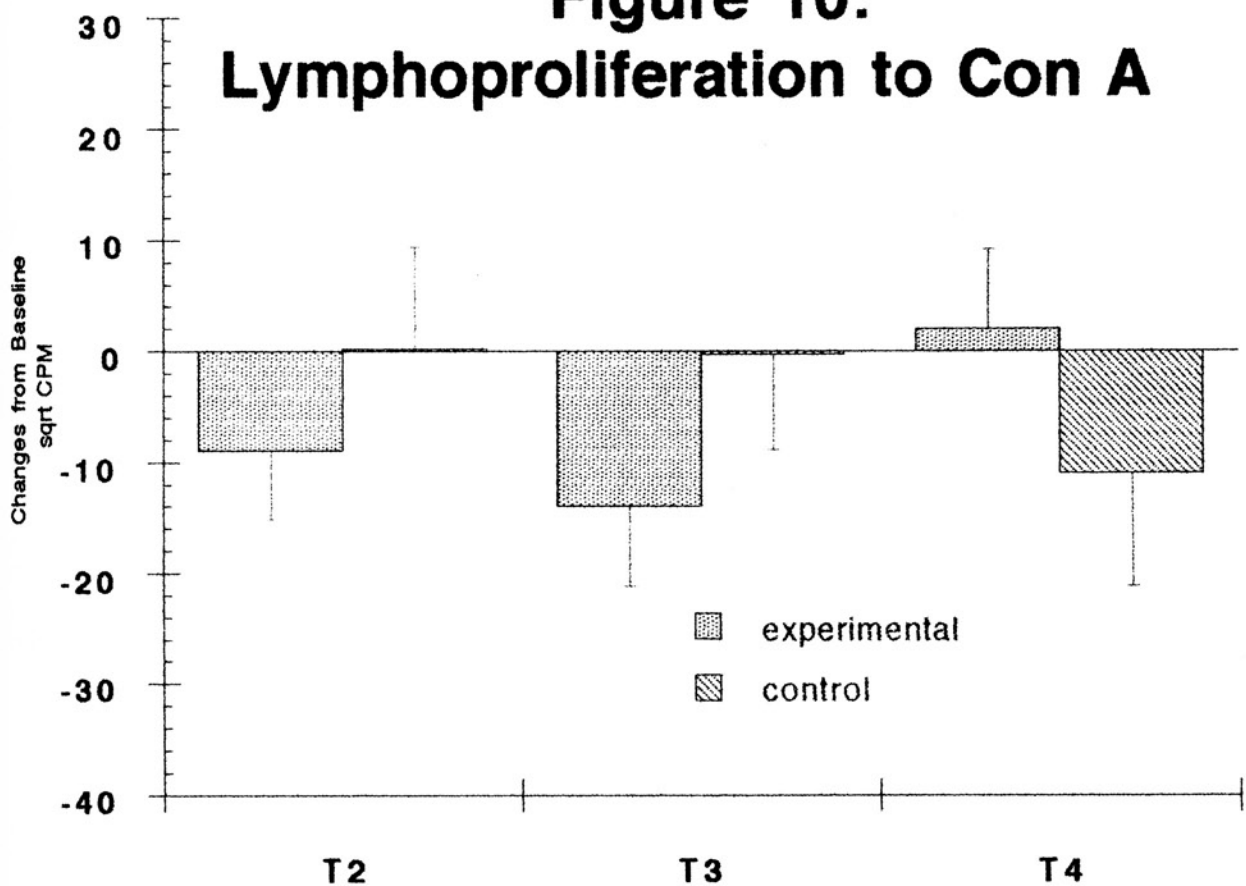


T2, post speech prep/reading to self; T3, 5 min. post speech/reading aloud; T4, 30 min. after speech/reading aloud; Groups, experimental (speech), control (reading); Sqrt cpm, squareroot of counts per minute.



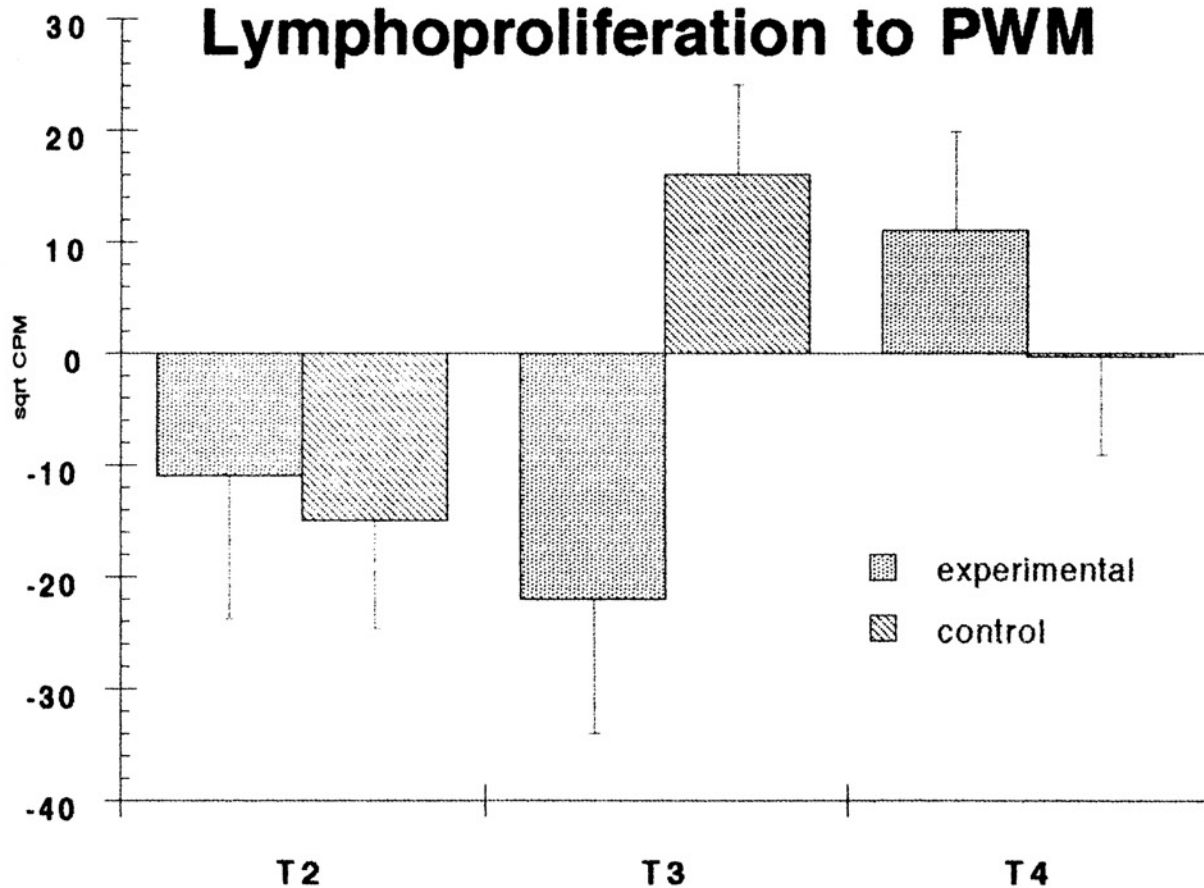
T2, after speech prep/reading to self; T3, 5 min. post speech/reading aloud; T4, 30 min. post speech/reading aloud; Groups, experimental (speech), control (reading); Sqrt CPM, squareroot of counts per minute.

Figure 10:
Lymphoproliferation to Con A



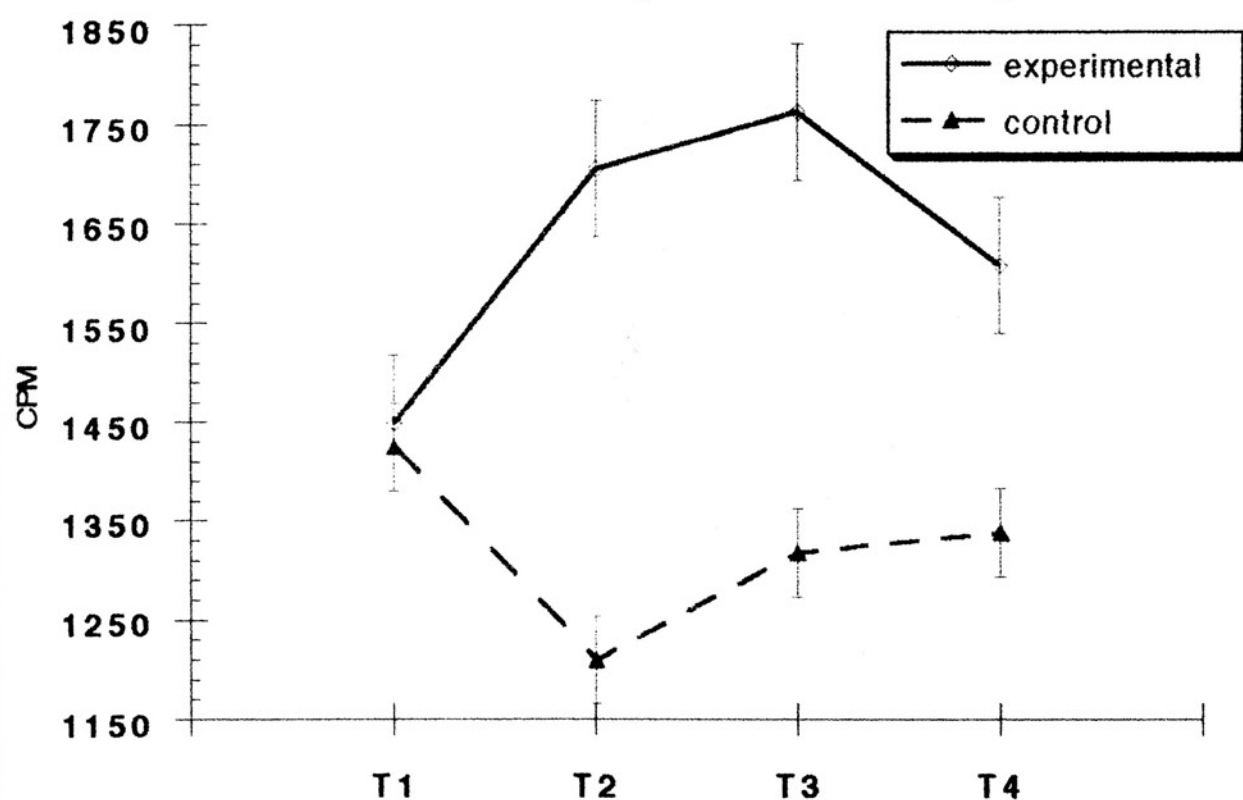
T2, post speech prep/reading to self; T3, 5 min. post speech/reading aloud; T4, 30 min. after speech/reading aloud; Groups, experimental (speech), control (reading); Sqrt cpm, squareroot of counts per minute.

Figure 11:
Lymphoproliferation to PWM



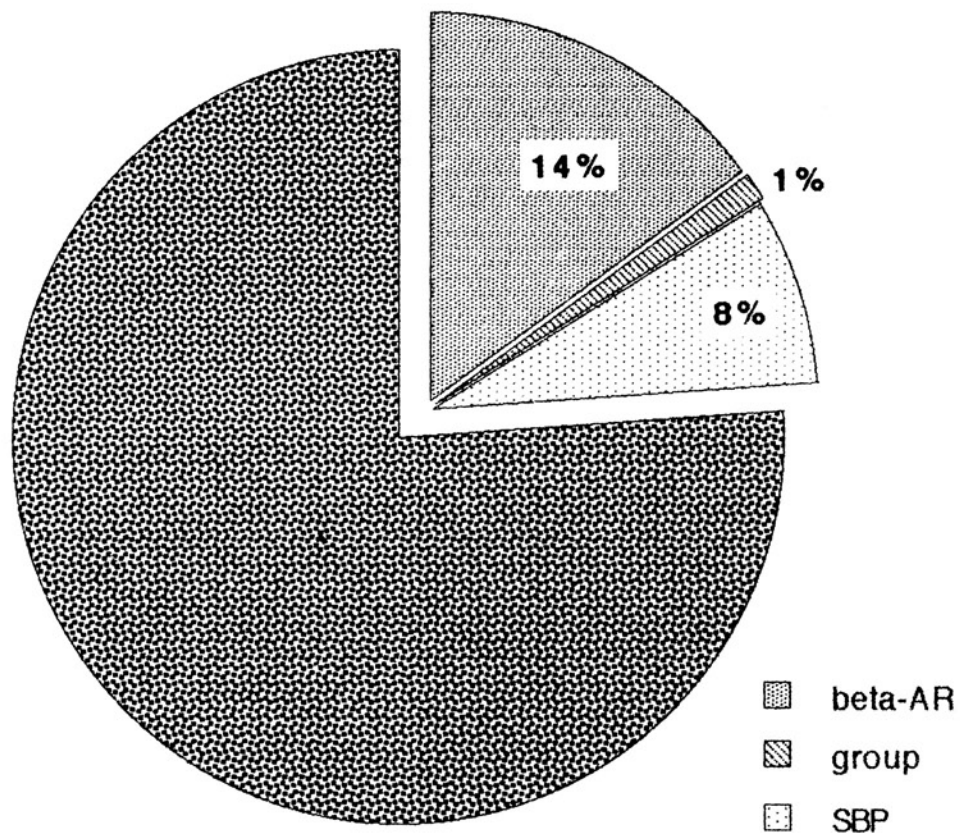
T2, after speech prep/reading to self; T3, 5 min. post speech/reading aloud; T4, 30 min. post speech/reading aloud; Groups, experimental (speech), control (reading); Sqrt CPM, squareroot of counts per minute.

Figure 12: Changes in Beta-adrenoreceptor Density



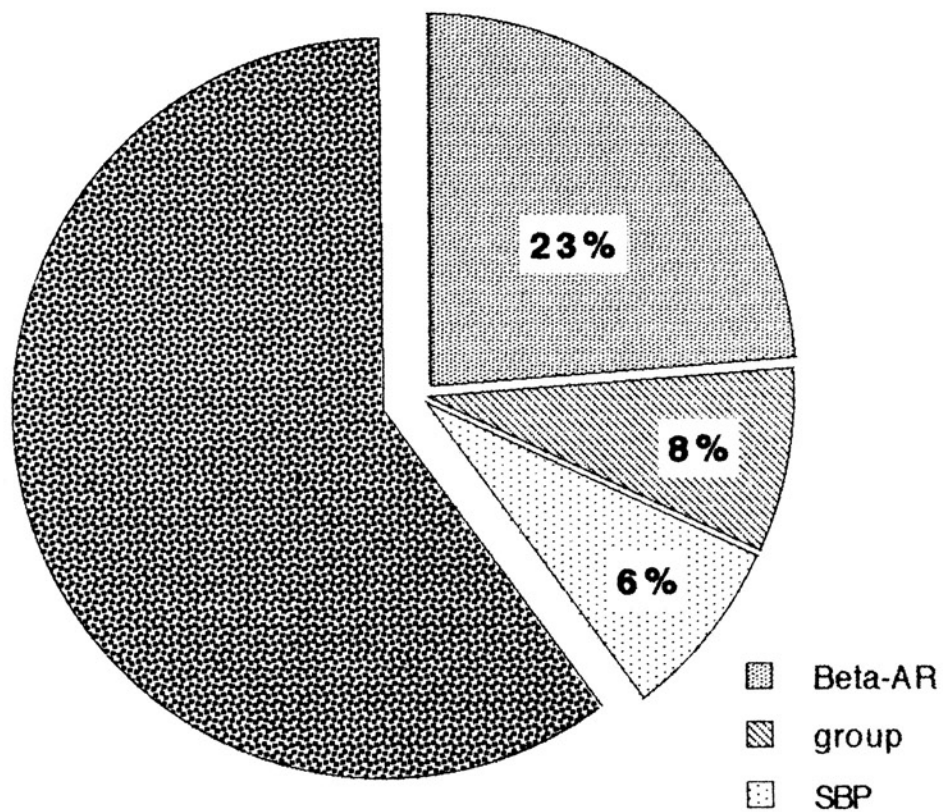
T1, baseline; T2, post speech prep./reading to self; T3, 5 min. post speech/reading aloud; T4, 30 min. post speech/reading aloud; Groups, experimental (speech), control (reading); CPM, counts per minute.

Figure 13: Con A



5 min. post speech/reading task (T3) changes in beta-adrenoreceptors (beta-AR), systolic blood pressure (SBP) and group predicted 14%, 8% and 1% of the variance for changes in proliferation to Con A from baseline.

Figure 14: PWM



5 min. post speech/reading task (T3) beta-adrenoreceptors (beta-AR), systolic blood pressure (SBP) and group predicted 23%, 6% and 8% of the variance respectively for proliferation to PWM from baseline.

Figure 15: Self Reports of Tension During the Tasks

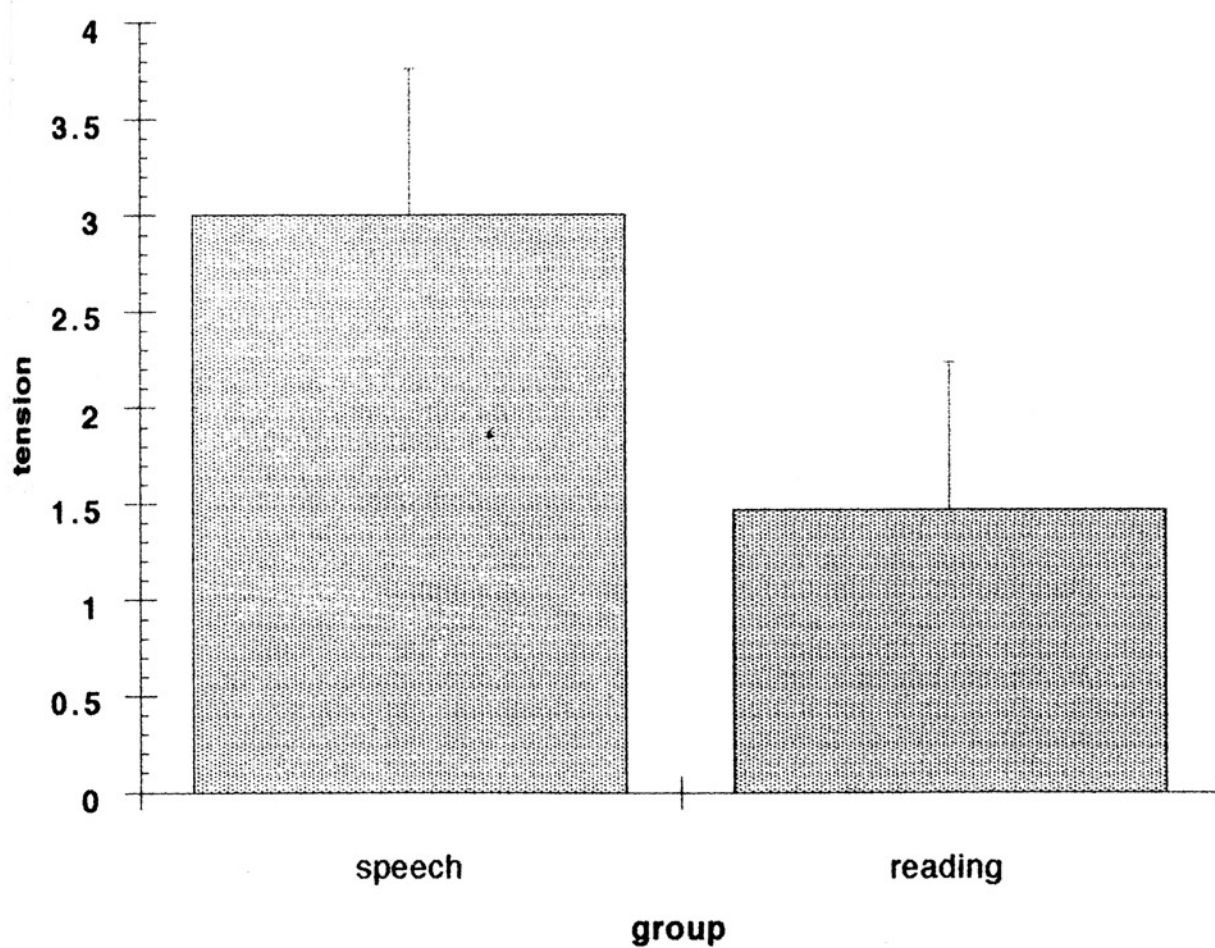
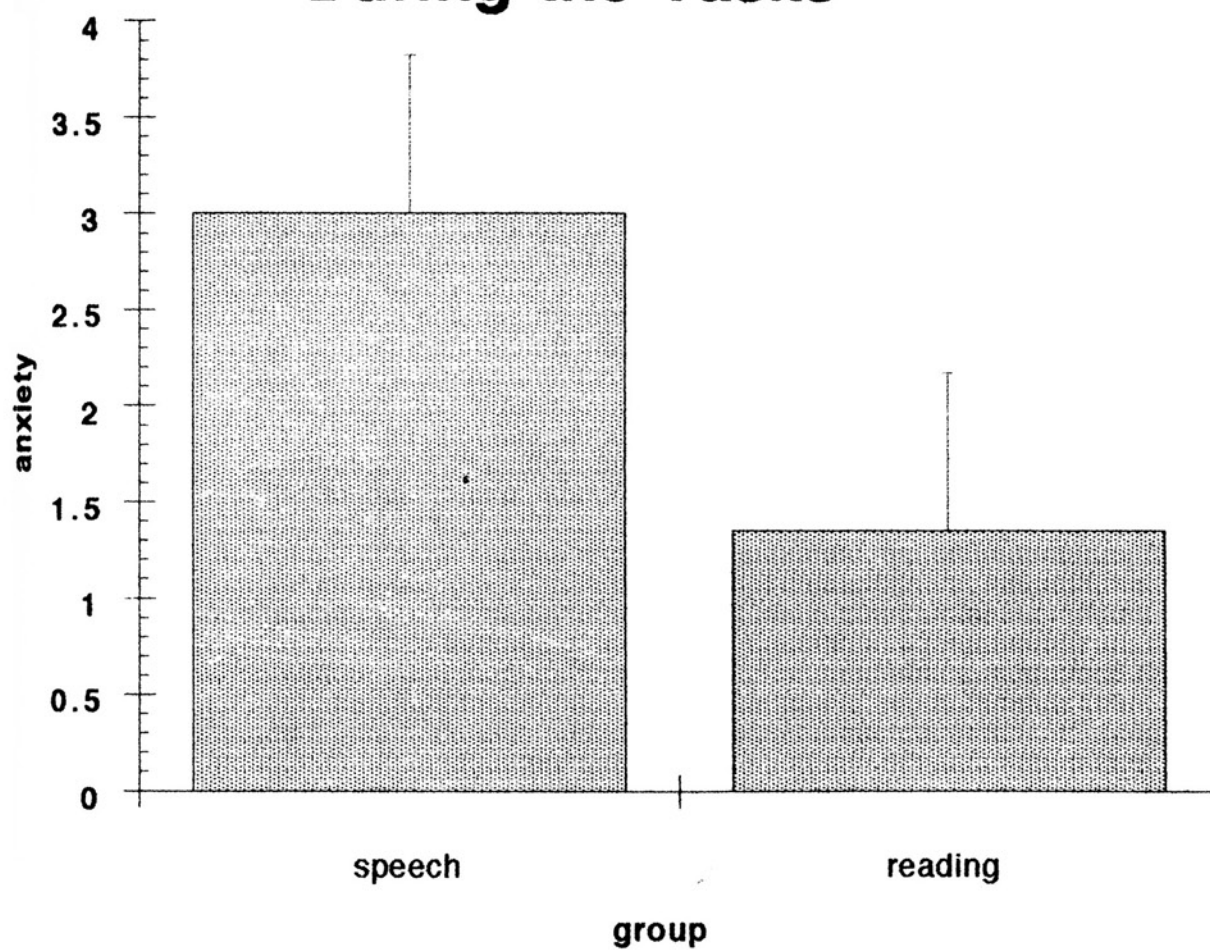


Figure 16: Self Reports of Anxiety During the Tasks



**Figure 17: Self Reports of Stress
During the Tasks**

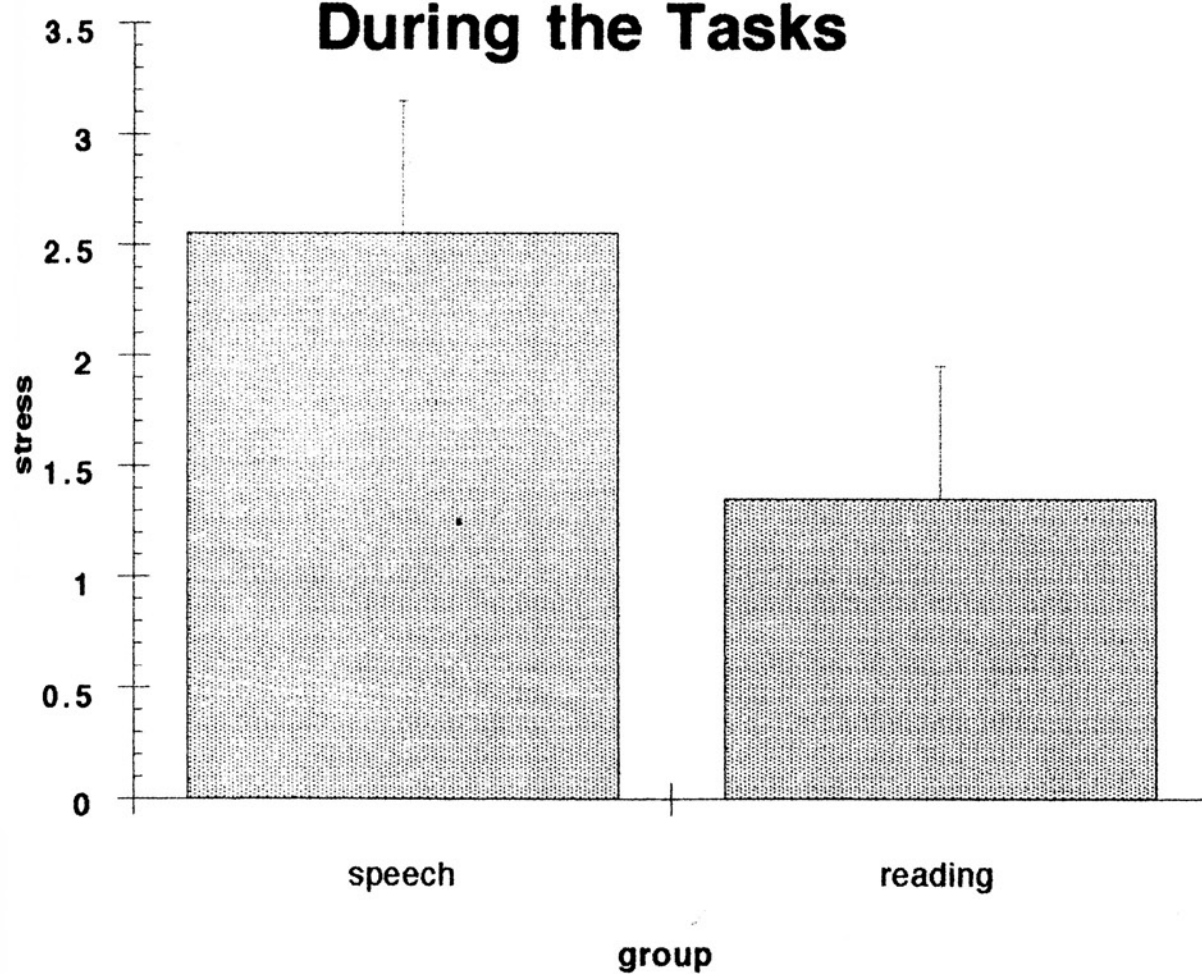


Table 1: Physiological Activity

	baseline	speech prep/ read to self	speech/ read aloud	recovery	Effect	F	p
SBP							
experimental	119 (11.2)	121 (9.9)	129 (12.8)*	117 (8.5)	Time	8.49	< .001
control	124 (11.1)	123 (12.9)	126 (13.8)	124 (12.1)	Group	.56	NS
					G X T	4.86	< .005
DBP							
experimental	73 (6.6)	78 (7.5)	85 (7.8)*	75 (6.5)	Time	14.62	< .001
control	81 (9.9)	82 (11.7)	84 (11.7)	82 (9.2)	Group	.64	NS
					G X T	5.08	< .004
HR							
experimental	68 (10.5)	70 (9.7)	79 (15.1)*	66 (8.4)	Time	11.72	< .001
control	73 (9.8)	70 (9.8)	74 (10.9)	69 (8.7)	Group	.886	NS
					G X T	3.32	< .03

Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR);
 F value of a repeated measures analysis; For time and group X time (G X T)
 df = (3, 87); for group, df = (1,29). * $p < .05$ for changes from baseline.

Table 2: Mitogenic Responses to Con A and PWM

	Baseline	Speech prep./ reading to self	Speech/ reading aloud	Recovery	Effect	F	p
Con A					Time	.63	NS
experimental	306 (43.7)	297 (46.7)	292 (45.2)	307 (57.7)	Group	.11	NS
control	265 (71.8)*	265 (85.7)	265 (77.3)	254 (81.8)	G X T	3.79	< .03
PWM					Time	1.7	NS
experimental	177 (49.97)	167 (54.6)	155 (52.6)	188 (46.97)	Group	.02	NS
control	153 (57.2)	138 (64.8)	168 (49.96)*	152 (54.4)*	G X T	3.86	< .03

Lymphocyte response to pokeweed mitogen (PWM) and Concovalin A (Con A), plant lectins. For group X time ConA: $df = (2,60)$ & PWM: $df=(2,52)$.

F values for repeated measures, for time, and group X time (G X T).

Values are the differences in counts/minute (square root) between stimulated and unstimulated samples for PWM and Con A.

Mitogen concentrations for PWM were combined since no group X time X concentration effects were found. This was also the case for Con A.

* $p < .05$ group differences from previous row.

**Table 3: Cardiovascular Reactivity
and Lymphoproliferation**

	Group	Baseline	Speech prep./ reading to self	Speech/ reading aloud	Recovery
	high-	321(35.2)	304(32.6)	308(33.9)	322(48.2)
ConA: SBP	low-	292(45.7)*	289(61.5)	276(50.9)	295(54.5)
	control	265(71.8)*	265(85.7)	265(77.3)	254(81.8)
	high-	306(49.2)	278(50.7)	286(47.8)	305(60.3)
ConA: DBP	low-	306(37.5)	314(40.5)*	299(44.4)	312(45.5)
	control	265(71.8)*	265(85.7)	264(77.3)	254(81.8)
	high-	304(48.1)	286(53.8)	292(50.4)	309(62.1)
ConA: HR	low-	308(38.8)	306(43.0)	292(42.6)	308(43.4)
	control	265(71.8)*	265(85.7)	265(77.3)	254(81.8)
	high-	192(27)	158(64.0)	178(59.1)	171(29.2)
PWM : SBP	low-	157(47.1)*	158(45.4)*	125(44.1)	201(42.3)*
	control	153(57.2)	138(64.8)	168(50.0)*	152(54.4)*
	high-	169(35.7)	140(55.1)	142(28.5)	186(28.3)
PWM: DBP	low-	179(48.4)	174(49.7)	161(77.8)	185(48.5)
	control	153(57.2)*	138(64.8)	168(50.0)*	152(54.4)
	high-	173(45.1)	166(55.3)	164(62.6)	207(30.3)
PWM: HR	low-	175(40.5)	148(54.0)	139(52.9)	165(35.2)*
	control	153(57.2)	138(64.8)	168(50.0)*	152(54.4)*

Mean lymphoproliferation to PWM and Con A, among high and low- reactive stress subjects and unstressed controls (standard deviations in parentheses).

* $p < .05$ group differences from previous row or rows.

APPENDIX I

B-adrenoreceptor Binding Assay

Lymphocyte Preparation:

1. Put 5 mls of 1 M EDTA into a 10 ml conical centrifuge tube and put in 10 mls of whole blood; when collecting blood, should be anti-coagulated.
2. Pipet whole blood very slowly over 4 mls Ficoll. DO NOT MIX.
3. Spin tubes at 1400 rpm for 30 minutes (approximately 400 g on table top centrifuge) at 4°C.
4. Collect lymphocyte layer with a pipet and put in 50 ml tube.
5. Fill to 10 mls with cold PBS (* make sure the transferred lymphocyte layer is diluted at least 2 fold).
6. Spin at 1200 rpm for 15 minutes; invert tube and pour off supernatant -- DO TWICE.
11. Bring up to 2 mls with DME-H+BSA and count with a hemocytometer.
12. Dilute to 2×10^6 cells/ml with DME-H+BSA

Binding of $^{125}\text{IPIND}$ to B-Adrenoreceptors:

The following reagents will be added to 10 ml plastic tubes:

.025 ml of $^{125}\text{IPIND}$ in each tube, range from 250 pM to 6 pM

(final) 0.25 ml of buffer, 1 μM (-) propranolol, or 1 μM CGP-12177

.200 ml of cells 2×10^6 total

1) Put cells in last, cover tubes with parafilm, shake and put in a radiation posted refrigerator for 2 days.

2) Using the Brandell Harvester add 10 mls of ice cold .1X PBS

(dilute PBS 1:10 with dH₂O) let sit for 5 minutes.

3) Cells will be harvested using PBS as wash buffer, and $^{125}\text{IPIND}$ will be bound to Whatson GF/C filter paper.

4) The $^{125}\text{IPIND}$ bound to filter paper will be placed in scintillation vials containing scintillation fluid and counted in a Gamma radiation counter.

* the cell harvester will have a swipe test performed with filter paper after each use and the filter paper will be placed in scintillation fluid and counted with the rest of the samples.

APPENDIX II

T- and B- Lymphocyte Proliferation Assay, Using ConA and PWM

- 1) Wipe counter with alcohol, turn on light and blower.
- 2) 4 mls of Ficoll Hypaque + 7 mls of blood layered on top in 15 ml conical tube (polypropylene so it will not break).
- 3) Centrifuge at 1500-2000 rpm for 30 minutes, at 25 degrees C, with the break off!
- 4) In the mean time, pipet 100 uls of ConA and PWM into appropriate wells.
- 5) After centrifuging for 30 minutes the layers are:
 - Plasma
 - Buffy Coat: Lymphocytes
 - Ficoll
 - Red Blood Cells
- 5) Pipet out lymphocyte layer and resuspend it with RPMI to 15 mls.
- 6) Centrifuge for 10 minutes with the break turned on!
- 7) Pour off supernatant and resuspend to 15 mls, and dissolve pellet.
- 8) Centrifuge for 10 minutes with the break turned on.

- 9) Pour off supernatant and resuspend to 2 mls, and dissolve pellet.
- 10) Count cells: make a 1:40 dilution with Trypan Blue, which stains dead cells (eg. 20 uls of cells and 280 uls of stain).
- 11) Dilute cells to 2×10^6 with RPMI.
 - a. calculation for number of cell you have:
cells counted X dilution factor with stain (40) X hemocytometer
 - b. calculations for diluting to number of cells you need:
 - for 3 mls of cell solution, make up 6×10^6 cells
 - divide 6×10^6 cells/ # cells you have (eg. 8.4×10^6) =
714 ul of cells
 - so 2.3 mls of RPMI would be needed
- 12) Pipet 100 uls of cells into each well that already has 100 uls of mitogen.
- 13) Incubate at 37° C and 5% CO₂ for 48 hours.
- 14) Pipet 20 uls of tritiated thymidine to each well.
- 15) Harvest cells 18 hours later with cell harvester.
- 16) Put 4 mls of scintillation fluid into each vial containing filter disk with cells and wait 24 hours.
- 17) Count β -emodin with a β -counter.

APPENDIX III: Questionnaires

Background Data

1. How old are you? _____

2. What is your occupation? _____

What is your spouse's occupation? _____

3. Approximate annual income: _____ Under \$10,000/year
 _____ \$10,000 - \$15,000/year
 _____ \$15,001 - \$20,000/year
 _____ \$20,001 - \$30,000/year
 _____ \$30,001 - \$40,000/year
 _____ \$40,001 - \$50,000/year
 _____ over \$50,000/year

4. How would you characterize where you live now?

_____ Rural Community
_____ Small Town
_____ Suburban Neighborhood
_____ Urban Neighborhood
_____ Other (Specify)

5. How long have you lived at your present residence? _____

6. What is your marital status?

_____ Single
 _____ Married How long? _____
 _____ Separated How long? _____
 _____ Divorced How long? _____
 _____ Widowed How long? _____

7. If you were previously married, how long were you married? _____

8. Number of family members living within 30 miles? _____

9. Your highest education level?

_____ Grammar School
 _____ High School
 _____ Some college
 _____ College Degree
 _____ Graduate Work
 _____ Other (specify) _____

10. Number of people living at your residence? _____

Are any of these people not relatives? yes _____ no _____

If yes, specify relationship _____

Food Checklist

Please circle any of the following items which you consumed during the past 15 hour period.

Coffee number of cups _____

Tea number of cups _____

Cola number of cups _____

Chocolate, cocoa, wine, beer/alcohol, decaffeinated coffee.

Breads containing raisins, prunes, orange peel, banana or pineapple.

Cheese bread, nut bread containing walnuts.

Raisin bran.

Desserts containing walnuts, sour cream or fruits, such as fruit cake, plum pudding, mince pie.

Banana, avocado, pineapple, canned figs, raisins, plums and prunes.

Oranges, orange juice, fruit cocktail with pineapple.

Tomato, broad beans (fava beans), eggplant or any vegetable in cheese sauce.

Chicken liver, herring, smoked or pickled fish, brain, aged cheese, sour cream, anchovies.

Cheese omelets, spanish omelets with aged cheese.

Macaroni and cheese, spaghetti in tomato sauce.

Walnuts, chocolate or coffee flavored candy, candy containing walnuts.

Catsup, chili sauce, olives, vanilla.

Do you smoke?

yes

no

Please list any medications that you are currently taking:

Profile of Moods Prior to the Task

Below is a list of words that describe feelings people have. Please read each carefully and check the box best describes how you feel now.

n o t a t a l l	a l i t t l e	m o d e r a t e l y	q u i t e a b i t	e x t r e m e l y		n o t a t a l l	a l i t t l e	m o d e r a t e l y	q u i t e a b i t	e x t r e m e l y	
					friendly						relaxed
					tense						bewildered
					happy						sluggish
					angry						uneasy
					worn out						kindly
					unhappy						lonely
					confused						miserable
					lively						efficient
					unable to concentrate						bitter
					sorry for things done						pleased
					shakey						alert
					listless						ready to fight
					overjoyed						restless
					peevd						good-natured
					agreeable						gloomy

Profile of Moods During the Task

Below is a list of words that describe feelings people have. Please read each carefully and check the box that best describes how you felt during the task.

n o t a t a l l	a l i t t l e	m o d e r a t e l y	q u i t e a b i t	e x t r e m e l y		n o t a t a l l	a l i t t l e	m o d e r a t e l y	q u i t e a b i t	e x t r e m e l y	
					friendly						relaxed
					tense						bewildered
					happy						sluggish
					angry						uneasy
					worn out						kindly
					unhappy						lonely
					confused						miserable
					lively						efficient
					unable to concentrate						bitter
					sorry for things done						pleased
					shakey						alert
					listless						ready to fight
					overjoyed						restless
					peevd						good-natured
					agreeable						gloomy

Profile of Moods During the Task

Below is a list of words that describe feelings people have. Please read each carefully and check the box that best describes how you felt during the task.

n o t a t a l l	a l i t t l e	m o d e r a t e l y	q u i t e a b i t	e x t r e m e l y		n o t a t a l l	a l i t t l e	m o d e r a t e l y	q u i t e a b i t	e x t r e m e l y	
					friendly						relaxed
					tense						bewildered
					happy						sluggish
					angry						uneasy
					worn out						kindly
					unhappy						lonely
					confused						miserable
					lively						efficient
					unable to concentrate						bitter
					sorry for things done						pleased
					shakey						alert
					listless						ready to fight
					overjoyed						restless
					peevd						good-natured
					agreeable						gloomy

Public Speaking Questionnaire

1. I look forward to an opportunity to speak in public.
2. My hands tremble when I try to handle objects on the platform.
3. I am in constant fear of forgetting my speech.
4. Audiences seem friendly when I address them.
5. While preparing a speech, I am in a constant state of anxiety.
6. At the conclusion of a speech, I feel that I have had a pleasant experience.
7. I dislike to use my body and voice expressively.
8. My thoughts become confused and jumbled when I speak before an audience.
9. I have no fear of facing an audience.
10. Although I am nervous just before getting up, I soon forget my fears and enjoy the experience.
11. I face the prospect of making a speech with complete confidence.
12. I feel that I am in complete possession of myself while speaking.
13. I prefer to have notes on the platform in case I forget my speech.
14. I like to observe the reactions of the audience to my speech.
15. Although I talk fluently with friends, I am at a loss for words on the platform.
16. I feel relaxed and comfortable while speaking.
17. Although I do not enjoy speaking in public, I do not particularly dread it.
18. I always avoid speaking in public if possible.
19. The faces of my audience are blurred when I look at them.
20. I feel disgusted with myself after trying to address a group of people.
21. I enjoy preparing a talk.

- 22. My mind is clear when I face an audience.
- 23. I am fairly fluent.
- 24. I perspire and tremble just before getting up to speak.
- 25. My posture feels strained and unnatural.
- 26. I am fearful and tense all the while I am speaking before a group of people.
- 27. I find the prospect of speaking mildly pleasant.
- 28. It is difficult for me to calmly search my mind for the right words to express my thoughts.
- 29. I am terrified at the thought of speaking before a group of people.
- 30. I have a feeling of alertness in facing an audience.

Recent Life Changes Questionnaire

I. Instructions for marking your recent life changes

To answer the questions below mark an "X" in one or more of the columns to the right of each question. If the event in question has occurred to you within the past two years, indicate when it occurred by marking the appropriate column: 0-6 months ago, 7-12 months ago, etc. It may be the case with some of the events below that you experienced them over more than one of the time periods listed for the past two years. If so, mark all the appropriate columns. If the event has not occurred to you during the last two years (or has never occurred to you) leave all the columns empty.

Now go through the questionnaire and mark your recent life changes. The column marked "Your Adjustment Score" will be explained at the end of the questionnaire.

A. Health: within the time periods listed, have you experienced:

	19-24 mo. ago	13-18 mo. ago	7-12 mo. ago	0-6 mo. ago	your adjustment score
1. an illness or injury which: a) kept you in bed a week or more, or took you to the hospital?					
b) was less serious than described above?					
2. a major change in eating habits?					
3. a major change in sleeping habits?					
4. a change in your usual type and/or amount of recreation?					
5. major dental work?					

B. Work: within the time periods listed, have you experienced:

	19 - 24 mo. ago	13 - 18 mo. ago	7-12 mo. ago	0 - 6 mo. ago	your adjustment score
6. Changed to a new type of work?					
7. changed your work hours or conditions?					
8. had a change in your responsibilities at work?					
a) more responsibilities					
b) less responsibilities?					
c) promotion?					
d) demotion?					
e) transfer?					
9. experienced troubles at work?					
10. experienced a major business readjustment?					
11. retired?					
12. experienced being:					
a) fired from work?					
b) laid off from work?					
13. taken courses by mail or studied at home to help you in your work?					

C. Home and Family: within the time periods listed, have you experienced:

	19 - 24 mo. ago	13 - 18 mo. ago	7-12 mo. ago	0 - 6 mo. ago	your adjustment score
14. a change in residence:					
a) a move within the same town or city?					

b) a move to a different town, city, or state?					
15. a change in family "get-togethers"?					
16. a major change in the health or behavior of a family member (illnesses, accidents, drug or disciplinary problems, etc.)?					
17. the death of a spouse?					
18. the death of a:					
a) child?					
b) brother or sister?					
c) parent?					
d) other close family member?					
19. the death of a close friend?					
20. a change in the marital status of your parents:					
a) divorce?					
b) remarriage?					

note: (Questions 21-32 concern marriage. For persons never married, go to item 33)

21. marriage?					
22. a change in arguments with your spouse?					
23. in-law problems?					
24. a separation from spouse:					
a) due to work?					

b) due to marital problems?					
25. reconciliation with spouse?					
26. a divorce?					
27. a gain of a new family member:					
a) birth of a child?					
b) adoption of a child?					
c) a relative moving in with you?					
28. spouse beginning or ceasing work outside the home?					
29. wife becoming pregnant?					
30. a child leaving home:					
a) due to marriage?					
b) to attend college?					
c) for other reasons?					
31. wife having a miscarriage or an abortion?					
32. birth of a grandchild?					

D. Personal and Social: within the time periods listed, have you experienced:

	19-24 mo. ago	13-18 mo. ago	7-12 mo. ago	0-6 mo. ago	your adjustment score
33. a major personal achievement?					

34. a change in your personal habits (your dress, friends, life-style, etc.					
35. sexual difficulties?					
36. beginning or ceasing school or college?					
37. a vacation?					
38. a change in your religious beliefs?					
39. a change in your social activities (clubs, movies, visiting)?					
40. a change of school or college?					
41. a minor violation of the law?					
42. legal troubles resulting in your being held in jail?					
43. a change in your political beliefs?					
44. a new, close, personal relationship?					
45. an engagement to marry?					
46. a "falling out" of a close personal relationship?					
47. girlfriend (or boyfriend) problems?					
48. a loss or damage of personal property?					
49. an accident?					

50. a major decision regarding your immediate future?					
---	--	--	--	--	--

E. Financial: within the time periods listed, have you experienced:

	19-24 mo. ago	13-18 mo. ago	7-12 mo. ago	0-6 mo. ago	your adjustment score
51. taken on a moderate purchase, such as a T.V., car, freezer?					
52. taken on a major purchase or a mortgage loan, such as a home, business, property?					
53. experienced a foreclosure on a mortgage or loan?					
54. experienced a major change in finances:					
a) increased income?					
b) decreased income?					
c) credit rating difficulties?					

Instructions for Scoring Your Adjustment to Your Recent Life Changes

Persons adapt to their recent life changes in different ways. Some people find the adjustment of a residential move, for example, to be enormous, while others find very little life adjustment necessary. You are now requested to "score" each of the recent life changes that you marked with an "X" as to the amount of adjustment you needed to handle the event.

Your scores can range from 1-100 "points". If, for example, you experienced a recent residential move but felt it required very little life adjustment, you would choose a low number and place it in the blank to the right of the question's box. On the other hand, if you recently changed residence and felt it required a near maximal life adjustment, you would place a high number, toward 100, in the blank to the right of that

question's box. For intermediate life adjustment scores you would choose intermediate numbers between 1 and 100.

Please go back through your questionnaire and for each recent life change you indicated with an "X", choose your personal life change adjustment score (between 1 and 100) which reflects what you saw to be the amount of life adjustment necessary to cope with or handle the event. Use both your estimates of the intensity of the life change and its duration to arrive at your scores.

6. I felt aroused/excited:

<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
(1)	(2)	(3)	(4)	(5)
not at all				very much

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